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(54) Title: CYTOCHROME P-450 CONSTRUCTS AND METHODS OF PRODUCING HERBICIDE-RESISTANT TRANSGENIC PLANTS

(57) Abstract

DNA sequence encoding novel cytochrome P-450 molecules are provided. The use of DNA constructs containing such molecules to transform plants is described, as are transgenic plants exhibiting increased resistance to phenylurea herbicides. Methods of using such DNA constructs and transformed plants are provided.

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NOVEL CYTOCHROME P-450 CONSTRUCTS AND METHODS OF PRODUCING HERBICIDE-RESISTANT TRANSGENIC PLANTS

Field of the Invention

The present invention relates to DNA encoding novel cytochrome P-450 molecules, and the transformation of cells with such DNA. These DNA sequences may be used in methods of producing plants with an altered ability to metabolize chemical compounds, such as phenylurea herbicides.

Background of the Invention

Cytochrome P-450 (P-450) monooxygenases are ubiquitous hemoproteins present in microorganisms, plants and animals. Comprised of a large and diverse group of isozymes, P-450s mediate a great array of oxidative reactions using a wide range of compounds as substrates, and including biosynthetic processes such as phenylpropanoid, fatty acid, and terpenoid biosynthesis; metabolism of natural products; and detoxification of foreign substances (xenobiotics). *See e.g.*, Schuler, *Crit. Rev. Plant Sci.* 15:235-284 (1996). In a typical P-450 catalyzed reaction, one atom of molecular oxygen (O_2) is incorporated into the substrate, and the other atom is reduced to water by NADPH. For most eucaryotic P-450s, NADPH:cytochrome P-450 reductase, a membrane-bound flavoprotein, transfers the necessary two electrons from NADPH to the P-450 (Bolwell et al, *Phytochemistry* 37: 1491-1506 (1994)).

Frear et al. (Phytochemistry 8:2157-2169 (1969)) demonstrated the metabolism of monuron by a mixed-function oxidase located in a microsomal fraction of cotton seedlings. Further evidence has accumulated supporting the involvement of P-450s in the metabolism and detoxification of numerous herbicides representing several distinct classes of compounds (reviewed in Bolwell et al., 1994; Schuler, 1996). Differential herbicide metabolizing P-450 activities are believed to represent one of the mechanisms that enables certain crop species to be more tolerant of a particular herbicide than other crop or weedy species.

Summary of the Invention

A first aspect of the present invention is an isolated DNA molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:17; or DNA sequences which encode an enzyme of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO6:, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18; or DNA sequences which have at least about 90% sequence identity to the above DNA and which encode a cytochrome P450 enzyme; and DNA sequences which differ from the above DNA due to the degeneracy of the genetic code.

A further aspect of the present invention is a cytochrome p450 enzyme having an amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO6:, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.

A further aspect of the present invention is an isolated DNA molecule comprising SEQ ID NO:1; DNA sequences which encode an enzyme of SEQ ID NO:2,; DNA sequences which have at least about 90% sequence identity to the above DNA and which encode a cytochrome P450 enzyme; and DNA sequences which differ from the above DNA due to the degeneracy of the genetic code.

A further aspect of the present invention is a cytochrome p450 peptide of SEQ ID NO:2.

A further aspect of the present invention is a DNA construct comprising a promoter operable in a plant cell and a DNA segment encoding a peptide of SEQ ID NO:2 downstream from and operatively associated with the promoter.

A further aspect of the present invention is a method of making a transgenic plant cell having an increased ability to metabolize phenylurea compounds compared to an untransformed plant cell. The plant cell is transformed with an exogenous DNA construct comprising a promoter operable in a plant cell and a DNA sequence encoding a peptide of SEQ ID NO:2. Transformed plants, seed and progeny of such plants are also aspects of the

present invention.

A further aspect of the present invention is a transgenic plant having an increased ability to metabolize phenylurea compounds. Such transgenic plants contain exogenous DNA encoding a peptide of SEQ ID NO:2.

Brief Description of the Drawings

Figure 1 depicts dithionite-reduced carbon monoxide difference spectra, where the solid line represents microsomes isolated from yeast transformed with CYP71A10, and the dotted line shows the difference spectra from yeast transformed with control vector V-60. Microsomal protein concentration was 1 mg/ml.

Figure 2 shows thin-layer chromatograms of [^{14}C]-radiolabeled fluometuron, linuron, chlortoluron, and diuron and their respective metabolites after incubation of the radiolabeled herbicides with yeast microsomes containing the CYP71A10 protein. Initial substrate concentrations for fluometuron, linuron, chlortoluron and diuron were 5.2, 6.5, 4.0, and 3.7 μM, respectively. P = parent compound; M = metabolite.

Figure 3 shows the chemical structures of fluometuron, linuron, chlortoluron and diuron, and their previously characterized metabolites. The linuron and chlortoluron metabolites are designated major or minor depending on their predicted relative abundance in assays using yeast microsomes containing the soybean CYP71A10 protein.

Figure 4 shows thin-layer chromatograms using [^{14}C]-radiolabeled linuron in various control reactions. The complete reaction mixture (COMPLETE) contained 3.2 μM linuron, 0.75 mM NADPH and 2.5 mg/ml microsomal protein isolated from CYP71A10-transformed yeast in 50 mM phosphate buffer (pH 7.1). Other reactions varied from COMPLETE by the addition of carbon monoxide (+CO), the omission of NADPH (NO NADPH), or the use of yeast microsomes isolated from cells expressing the control vector (V-60). P = parent compound; M = metabolite.

Figure 5A shows tobacco line 25/2 plants (transformed with soybean CYP71A10) grown on media containing no herbicide.

Figure 5B shows control tobacco plants (transformed with vector pBI121) grown on media containing 0.5 μM linuron.

Figure 5C shows tobacco line 25/2 (transformed with soybean CYP71A10) individuals grown on media containing 0.5 μM linuron.

Figure 5D shows tobacco line 25/2 (transformed with soybean CYP71A10) individuals grown on media containing 2.5 μM linuron.

Figure 5E shows control tobacco plants (transformed with vector pBI121) grown on media containing 1.0 μM chlortoluron.

Figure 5F shows tobacco line 25/2 (transformed with soybean CYP71A10) individuals grown on media containing 1.0 μM chlortoluron.

Detailed Description of the Invention

1. Overview of the present research:

The present inventors utilized a strategy based on the random isolation and screening of soybean cDNAs encoding cytochrome P-450 (P-450) isozymes to identify P-450 isozymes involved in herbicide metabolism. Eight full-length and one near full-length P-450 cDNAs representing eight distinct P-450 families were isolated using polymerase chain reaction (PCR)-based technologies (**SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15** and 17). Five of these soybean P-450 cDNAs were successfully overexpressed in yeast, and microsomal fractions generated from these strains were tested for their potential to mediate the metabolism of ten herbicides and one insecticide. *In vitro* enzyme assays showed that the gene product of one heterologously expressed P-450 cDNA (CYP71A10) (**SEQ ID NO:1**) specifically mediated the metabolism of phenylurea herbicides, converting four herbicides of this class (fluometuron, linuron, chlortoluron, and diuron) into more polar metabolites. Analyses of the metabolites indicate that the CYP71A10 encoded enzyme functions primarily as an N-demethylase with regard to

fluometuron, linuron and diuron, and as a ring-methyl hydroxylase when chlortoluron is the substrate. *In vivo* assays using excised leaves demonstrated that all four herbicides were more readily metabolized in CYP71A10-transformed tobacco in comparison to control plants.

Shiota et al. reported that fused constructs derived from the rat CYP1A1 and yeast NADPH-cytochrome P-450 oxidoreductase cDNAs conferred chlortoluron resistance in tobacco by enhancing herbicide metabolism (Shiota et al., *Plant Physiol.* 106:17-23 (1994)). In another study, a chloroplast-targeted, bacterial CYP105A1 expressed in tobacco catalyzed the toxification of R7402, a sulfonylurea pro-herbicide (O'Keefe et al., *Plant Physiol.* 105:473-482 (1994)). The cloning and heterologous expression of an endogenous plant P-450 gene that is potentially involved in herbicide metabolism was reported by Pierrel et al., *Eur. J. Biochem.* 224:835-844 (1994), where a trans-cinnamic acid hydroxylase cDNA (CYP73A1) isolated from artichoke and expressed in yeast catalyzed the ring-methyl hydroxylation of chlortoluron. *In vivo* experiments with artichoke tubers, however, demonstrated that the ring-methyl hydroxy metabolite represented only a minor portion of the metabolites produced and that the major metabolite was demethylated chlortoluron (Pierrel et al., 1994). This together with the observation that the turnover number of the heterologously expressed enzyme was very low (0.014/ min), suggested that CYP73A1 plays a minimal role in chlortoluron metabolism *in vivo*. US Patent No. 5,349,127 to Dean et al. discloses the use of DNA encoding certain P-450 enzymes, isolated from *Streptomyces griseolus*, to produce transformed plants with increased metabolism of certain compounds. (All US patents referred to herein are intended to be incorporated herein in their entirety.)

Although the role of P-450 enzymes in catalyzing the metabolism of a variety of herbicides has been documented, little progress has been made in the identification of the endogenous plant P-450s that are responsible for degrading these compounds. Protein purification of specific isozymes involved in the metabolism of a specific herbicide has been hindered by the instability of the

enzymes, their low concentrations in most plant tissues, and difficulties in the reconstitution of active complexes from solubilized components. Furthermore, any given plant tissue may possess dozens, if not hundreds, of unique P-450 isozymes, complicating the purification to homogeneity of a particular isozyme. Because plants have only been exposed to phenylurea herbicides during the past few decades, it is unlikely that enzymes have evolved solely for the purposed of metabolizing this class of xenobiotics.

2. Use of CYP71A10 to produce phenylurea-resistant plants:

The present invention provides materials and methods useful in producing transgenic plant cells and plants with increased resistance to phenylurea herbicides. Increased herbicide resistance, as used herein, refers to the ability of a plant to withstand levels of an herbicide that have a negative impact on wild-type (untransformed) plants of the same species and/or variety. Resistance, as used herein, does not necessarily mean that the resistant plant is completely unaffected by exposure to the herbicide; rather, resistant plants suffer less extensive or less severe damage than comparable wild-type plants. Methods of assessing the extent and/or severity of herbicide impact will vary depending on the particular plant and the particular herbicide being tested; such assessment methods will be apparent to those skilled in the art. The negative effects of a herbicide may be evidenced by the complete arrest of plant growth, or by an inhibition in the rate or amount of growth. Additionally, methods of the present invention may be used to decrease herbicide residues in plants, even where the amounts of herbicides present in the plant do not cause an appreciable negative effect on the plant as a whole.

Increased resistance to a herbicide can be due to an increased ability to metabolize a herbicide to less harmful metabolites. Accordingly, plants of the present invention which exhibit increased resistance to a herbicide may also be described as having an increased ability to metabolize the starting herbicidal compound, where the metabolites are less harmful to the plant than the starting

compound.

In the examples provided herein, yeast microsomes and transgenic tobacco plants expressing the CYP71A10 peptide (**SEQ ID NO:2**) and exposed to various phenylurea herbicides produced the same degradation products that have previously been observed when these same compounds have been incubated with metabolically active plant microsomes. These results indicate that the CYP71A10 peptide plays a role in the effective metabolism of phenylurea herbicides.

The present examples demonstrate that the overexpression of a CYP71A10 peptide of **SEQ ID NO:2** in tobacco enhanced the plant's capacity to metabolize all four phenylurea herbicides tested, and that appreciable levels of tolerance were conferred to linuron and chlortoluron. Fluometuron was the most actively metabolized compound in both the yeast and transgenic plant systems, yet the enhancement in tolerance to this herbicide at the whole plant level was not as great as for linuron and chlortoluron. While not wishing to be held to a single theory, the present inventors surmise that the lack of correlation between the rate of herbicide metabolism and herbicide tolerance may be explained by the differential toxicities of the various phenylurea derivatives produced in the CYP71A10-transformed tobacco. Consistent with this hypothesis are the previous observations that N-demethyl derivatives of fluometuron, diuron and chlortoluron are only moderately less toxic than their parent compounds (Rubin and Eshel, *Weed Sci.* 19:592-594 (1971); Dalton et al., *Weeds* 14:31-33 (1966); Ryan and Owen, *Proc. Brit. Crop Prot. Conf. Weeds* 1:317-324 (1982)). In contrast, linuron is a 10-fold greater inhibitor of the Hill-reaction than N-demethyl linuron (Suzuki and Casida, *J. Agric. Food Chem.* 29:1027-1033 (1981)), and the hydroxylated and the didemethlayed derivatives of chlortoluron are considered to be nonherbicidal (Ryan and Owen, 1982).

The present inventors found that the relative rates of herbicide metabolism in leaves of CYP71A10-transformed tobacco and in yeast microsomes assayed *in vitro* were similar (see **Tables 4** and **5**). With the exception of the transgenic

plant leaves showing a somewhat greater metabolic activity against chlortoluron than was apparent in the yeast microsomal assays, both systems followed the general order of metabolism of fluometuron $\geq$ linuron > chlortoluron > diuron. These results indicate that expression of a test plant P-450 in yeast and quantification of the metabolism of a test compound using yeast microsomes, is a suitable system for screening plant P-450s for their metabolic function, and for their potential usefulness in the production of transgenic plants with altered metabolism of chemical compounds such as herbicides and insecticides.

The present inventors have shown that the random isolation of P-450 cDNAs with subsequent heterologous expression in yeast is an effective strategy to characterize cDNAs whose product is capable of affecting the metabolism of a test compound. This approach is useful in characterizing the substrates (both natural and artificial) affected by a P-450, in determining the function of P-450 genes whose catalytic activities remain unclear, and in screening P-450s for the ability to increase or decrease the metabolism of a test compound. A particularly useful aspect of this method is the ability to screen isolated P-450s for their effects on the metabolism by plants of herbicides, insecticides, or other chemical compounds. Increased metabolism may result in enhanced resistance to the effects of a compound (where the metabolites are less harmful than the starting compound), or in increased sensitivity to the effects of a compound (where one or more metabolites are more toxic than the starting compound; *see* O'Keefe et al., 1994).

3. DNA Constructs:

Those familiar with recombinant DNA methods available in the art will recognize that one can employ a cDNA molecule (or a chromosomal gene or genomic sequence) encoding a P-450 peptide, joined in the sense orientation with appropriate operably linked regulatory sequences, to construct transgenic cells and plants. (Those of skill in the art will also recognize that appropriate regulatory sequences for expression of genes in the sense orientation include any

one of the known eukaryotic translation start sequences, in addition to the promoter and polyadenylation/transcription termination sequences described herein). Appropriate selection of the encoded P-450 peptide will provide transformed plants characterized by altered (enhanced or retarded) metabolism of phenylurea compounds.

DNA constructs, or "transcription cassettes," of the present invention include, 5' to 3' in the direction of transcription, a promoter as discussed herein, a DNA sequence as discussed herein operatively associated with the promoter, and, optionally, a termination sequence including stop signal for RNA polymerase and a polyadenylation signal for polyadenylase. All of these regulatory regions should be capable of operating in the cells of the tissue to be transformed. Any suitable termination signal may be employed in carrying out the present invention, examples thereof including, but not limited to, the nopaline synthase (nos) terminator, the octapine synthase (ocs) terminator, the CaMV terminator, or native termination signals derived from the same gene as the transcriptional initiation region or derived from a different gene. See, e.g., Rezian et al. (1988) supra, and Rodermel et al. (1988), supra.

The term "operatively associated," as used herein, refers to DNA sequences on a single DNA molecule which are associated so that the function of one is affected by the other. Thus, a promoter is operatively associated with a DNA when it is capable of affecting the transcription of that DNA (i.e., the DNA is under the transcriptional control of the promoter). The promoter is said to be "upstream" from the DNA, which is in turn said to be "downstream" from the promoter.

The transcription cassette may be provided in a DNA construct which also has at least one replication system. For convenience, it is common to have a replication system functional in Escherichia coli, such as ColE1, pSC101, pACYC184, or the like. In this manner, at each stage after each manipulation, the resulting construct may be cloned, sequenced, and the correctness of the manipulation determined. In addition, or in place of the E. coli replication

system, a broad host range replication system may be employed, such as the replication systems of the P-1 incompatibility plasmids, e.g., pRK290. In addition to the replication system, there will frequently be at least one marker present, which may be useful in one or more hosts, or different markers for individual hosts. That is, one marker may be employed for selection in a prokaryotic host, while another marker may be employed for selection in a eukaryotic host, particularly the plant host. The markers may be protection against a biocide, such as antibiotics, toxins, heavy metals, or the like; may provide complementation, by imparting prototrophy to an auxotrophic host; or may provide a visible phenotype through the production of a novel compound in the plant.

The various fragments comprising the various constructs, transcription cassettes, markers, and the like may be introduced consecutively by restriction enzyme cleavage of an appropriate replication system, and insertion of the particular construct or fragment into the available site. After ligation and cloning the DNA construct may be isolated for further manipulation. All of these techniques are amply exemplified in the literature as exemplified by J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989)(Cold Spring Harbor Laboratory).

Vectors which may be used to transform plant tissue with nucleic acid constructs of the present invention include both Agrobacterium vectors and ballistic vectors, as well as vectors suitable for DNA-mediated transformation.

4. Promoters:

The term 'promoter' refers to a region of a DNA sequence that incorporates the necessary signals for the efficient expression of a coding sequence. This may include sequences to which an RNA polymerase binds but is not limited to such sequences and may include regions to which other regulatory proteins bind together with regions involved in the control of protein translation and may include coding sequences.

Promoters employed in carrying out the present invention may be constitutively active promoters. Numerous constitutively active promoters which are operable in plants are available. A preferred example is the Cauliflower Mosaic Virus (CaMV) 35S promoter which is expressed constitutively in most plant tissues. Use of the CaMV promoter for expression of recombinant genes in tobacco roots has been well described (Lam et al., "Site-Specific Mutations Alter In Vitro Factor Binding and Change Promoter Expression Pattern in Transgenic Plants", *Proc. Nat. Acad. Sci. USA* 86, pp. 7890-94 (1989); Poulsen et al. "Dissection of 5' Upstream Sequences for Selective Expression of the Nicotiana plumbaginifolia rbcS-8B Gene", *Mol. Gen. Genet.* 214, pp. 16-23 (1988)). In the alternative, the promoter may be a tissue-specific promoter or a promoter that is expressed temporally or developmentally. See, e.g., US Patent No. 5,459,252 to Conkling et al.; Yamamoto et al., *The Plant Cell*, 3:371 (1991). In methods of transforming plants to alter the effects of herbicides or to decrease residual amounts of herbicides or pesticides in plants, selection of a suitable promoter will vary depending on the plant species, the specific chemical compound used as a herbicide or pesticide, and the time and method of applying the chemical compound to the plant or plant crop, as will be apparent to those skilled in the art.

5. Selectable Markers:

The recombinant DNA molecules and vectors used to produce the transformed cells and plants of this invention may further comprise a dominant selectable marker gene. Suitable dominant selectable markers include, inter alia, antibiotic resistance genes encoding neomycin phosphotransferase (NPTII), hygromycin phosphotransferase (HPT), and chloramphenicol acetyltransferase (CAT). Another well-known dominant selectable marker suitable is a mutant dihydrofolate reductase gene that encodes methotrexate-resistant dihydrofolate reductase. DNA vectors containing suitable antibiotic resistance genes, and the corresponding antibiotics, are commercially available. Transformed cells are

selected out of the surrounding population of non-transformed cells by placing the mixed population of cells into a culture medium containing an appropriate concentration of the antibiotic (or other compound normally toxic to the untransformed cells) against which the chosen dominant selectable marker gene product confers resistance. Thus, only those cells that have been transformed will survive and multiply.

A further aspect of the present invention is use of the identified P-450 coding sequences as a selectable marker gene. A DNA construct comprising a sequence encoding a P-450 known to increase resistance to a compound (such as **SEQ ID NO:2)** is utilized to transform cells, in accordance with methods known in the art. Those cells that subsequently exhibit resistance to the compound are indicated as transformed. Such constructs may be used to verify the success of a transformation technique or to select transformed cells of interest.

6. Sequence similarity and hybridization conditions:

Nucleic acid sequences employed in carrying out the present invention include those with sequence similarity to **SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15** or **17**, and encoding a protein having P-450 enzymatic activity. This definition is intended to encompass natural allelic variants and minor sequence variations in the nucleic acid sequence encoding a P-450 molecule, or minor sequence variations in the amino acid sequence of the encoded product. Thus, DNA sequences that hybridize to DNA of **SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15** or **17** and code for expression of a P-450 enzyme, particularly a plant P-450 enzyme, may also be employed in carrying out aspects of the present invention. The nomenclature for P-450 genes is based on amino acid sequence identity; methods of determining sequence similarity are well-known to those skilled in the art. Typically, sequences sharing >40% identity are placed in the same family, >55% identity defines members of the same subfamily, and sequences that

display >97% identity are assumed to represent allelic variants. Conditions which permit other DNA sequences which code for expression of a protein having P-450 enzymatic activity to hybridize to DNA of **SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15** or **17**, or to other DNA sequences encoding the protein given as **SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16** or **18** can be determined in a routine manner. For example, hybridization of such sequences may be carried out under conditions of reduced stringency or even stringent conditions (e.g., conditions represented by a wash stringency of 0.3 M NaCl, 0.03 M sodium citrate, 0.1% SDS at 60°C or even 70°C to DNA encoding the protein given as **SEQ ID NO:2** herein in a standard in situ hybridization assay. See J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989)(Cold Spring Harbor Laboratory)). In general, such sequences will be at least 65% similar, 75% similar, 80% similar, 85% similar, 90% similar, 93% similar, 95% similar, or even 97% or 98% similar, or more, with the sequence given herein as **SEQ ID NO:1**, or DNA sequences encoding proteins of **SEQ ID NO:2**. (Determinations of sequence similarity are made with the two sequences aligned for maximum matching; gaps in either of the two sequences being matched are allowed in maximizing matching. Gap lengths of 10 or less are preferred, gap lengths of 5 or less are more preferred, and gap lengths of 2 or less still more preferred.)

As used herein, the term 'gene' refers to a DNA sequence that incorporates (1) upstream (5') regulatory signals including a promoter, (2) a coding region specifying the product, protein or RNA of the gene, (3) downstream (3') regions including transcription termination and polyadenylation signals and (4) associated sequences required for efficient and specific expression.

The DNA sequence of the present invention may consist essentially of a sequence provided herein (**SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15** or **17**), or equivalent nucleotide sequences representing alleles or polymorphic variants of these genes, or coding regions thereof.

Use of the phrase "substantial sequence similarity" in the present

specification and claims means that DNA, RNA or amino acid sequences which have slight and non-consequential sequence variations from the actual sequences disclosed and claimed herein are considered to be equivalent to the sequences of the present invention. In this regard, "slight and non-consequential sequence variations" mean that "similar" sequences (i.e., the sequences that have substantial sequence similarity with the DNA, RNA, or proteins disclosed and claimed herein) will be functionally equivalent to the sequences disclosed and claimed in the present invention. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same compositions as the nucleic acid and amino acid compositions disclosed and claimed herein.

DNA sequences provided herein can be transformed into a variety of host cells. A variety of suitable host cells, having desirable growth and handling properties, are readily available in the art.

Use of the phrase "isolated" or "substantially pure" in the present specification and claims as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been separated from their *in vivo* cellular environments through the efforts of human beings.

As used herein, a "native DNA sequence" or "natural DNA sequence" means a DNA sequence which can be isolated from non-transgenic cells or tissue. Native DNA sequences are those which have not been artificially altered, such as by site-directed mutagenesis. Once native DNA sequences are identified, DNA molecules having native DNA sequences may be chemically synthesized or produced using recombinant DNA procedures as are known in the art. As used herein, a native plant DNA sequence is that which can be isolated from non-transgenic plant cells or tissue.

7. Transformed plants:

Methods of making recombinant plants of the present invention, in general, involve first providing a plant cell capable of regeneration (the plant cell

typically residing in a tissue capable of regeneration). The plant cell is then transformed with a DNA construct comprising a transcription cassette of the present invention (as described herein) and a recombinant plant is regenerated from the transformed plant cell. As explained below, the transforming step is carried out by techniques as are known in the art, including but not limited to bombarding the plant cell with microparticles carrying the transcription cassette, infecting the cell with an Agrobacterium tumefaciens containing a Ti plasmid carrying the transcription cassette, or any other technique suitable for the production of a transgenic plant.

Numerous Agrobacterium vector systems useful in carrying out the present invention are known. For example, U.S. Patent No. 4,459,355 discloses a method for transforming susceptible plants, including dicots, with an Agrobacterium strain containing the Ti plasmid. The transformation of woody plants with an Agrobacterium vector is disclosed in U.S. Patent No. 4,795,855. Further, U.S. Patent No. 4,940,838 to Schilperoort et al. discloses a binary Agrobacterium vector (i.e., one in which the Agrobacterium contains one plasmid having the vir region of a Ti plasmid but no T region, and a second plasmid having a T region but no vir region) useful in carrying out the present invention.

Microparticles carrying a DNA construct of the present invention, which microparticle is suitable for the ballistic transformation of a plant cell, are also useful for making transformed plants of the present invention. The microparticle is propelled into a plant cell to produce a transformed plant cell, and a plant is regenerated from the transformed plant cell. Any suitable ballistic cell transformation methodology and apparatus can be used in practicing the present invention. Exemplary apparatus and procedures are disclosed in Sanford and Wolf, U.S. Patent No. 4,945,050, and in Christou et al., U.S. Patent No. 5,015,580. When using ballistic transformation procedures, the transcription cassette may be incorporated into a plasmid capable of replicating in or integrating into the cell to be transformed. Examples of microparticles suitable

for use in such systems include 1 to 5 μm gold spheres. The DNA construct may be deposited on the microparticle by any suitable technique, such as by precipitation.

Plant species may be transformed with the DNA construct of the present invention by the DNA-mediated transformation of plant cell protoplasts and subsequent regeneration of the plant from the transformed protoplasts in accordance with procedures well known in the art. Fusion of tobacco protoplasts with DNA-containing liposomes or via electroporation is known in the art. (Shillito et al., "Direct Gene Transfer to Protoplasts of Dicotyledonous and Monocotyledonous Plants by a Number of Methods, Including Electroporation", *Methods in Enzymology* 153, pp. 313-36 (1987)).

As used herein, transformation refers to the introduction of exogenous DNA into cells, so as to produce transgenic cells stably transformed with the exogenous DNA. Transformed plant cells are induced to regenerate intact plants through application of cell and tissue culture techniques that are well known in the art. The method of plant regeneration is chosen so as to be compatible with the method of transformation. The stable presence and the orientation of the exogenous DNA in transgenic plants can be verified by Mendelian inheritance of the DNA sequence, as revealed by standard methods of DNA analysis applied to progeny resulting from controlled crosses.

Plants of horticultural or agronomic utility, such as vegetable or other crops, can be transformed according to the present invention using techniques available in the art. A plant suitable for use in the present methods is *Nicotiana tabacum*, or tobacco. Any strain or variety of tobacco may be used. Additional plants (both monocots and dicots) which may be employed in practicing the present invention include, but are not limited to, potato (*Solanum tuberosum*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus spp.*)cassava (*Manihot esculenta*), coffee (*Cofea* spp.), pineapple (*Ananas comosus*), citrus trees (*Citrus*

spp.), banana (*Musa* spp.), corn (*Zea mays*), oilseed rape (*Brassica napus*), wheat, oats, barley, rye and rice. Thus, an illustrative category of plants which may be used to practice aspects of the present invention are the dicots, and a more particular category of plants which may be used to practice the present invention are members of the family Solanacae.

The methods of the present invention can further be practiced with turfgrass, including cool season turfgrasses and warm season turfgrasses. Examples of cool season turfgrasses are Bluegrasses (*Poa* L.), such as Kentucky Bluegrass (*Poa pratensis* L.), rough Bluegrass (*Poa trivialis* L.), Canada Bluegrass (*Poa compressa* L.), Annual Bluegrass (*Poa annua* L.), Upland Bluegrass (*Poa glaucantha* Gaudin), Wood Bluegrass (*Poa nemoralis* L.), and Bulbous Bluegrass (*Poa bulbosa* L.); the Bentgrasses and Redtop (*Agrostis* L.), such as Creeping Bentgrass (*Agrostis palustris* Huds.), Colonial Bentgrass (*Agrostis tenius* Sibth.), Velvet Bentgrass (*Agrostis canina* L.), South German Mixed Bentgrass (*Agrostis* L.), and Redtop (*Agrostis alba* L.); the Fescues (*Festuca* L.), such as Red Fescue (*Festuca rubra* L.), Chewings Fescue (*Festuca rubra* var. *commutata* Gaud.), Sheep Fescue (*Festuca ovina* L.), Hard Fescue (*Festuca ovina* var. *duriuscula* L. Koch), Hair Fescue (*Festuca capillata* Lam.), Tall Fescue (*Festuca arundinacea* Schreb.), Meadow Fescue (*Festuca elatior* L.); the Rye grasses (*Lolium* L.), such as Perennial Ryegrass (*Lolium perenne* L.), Italian Ryegrass (*Lolium multiflorum* Lam.); the Wheatgrasses (*Agropyron* Gaertn.), such as Fairway Wheatgrass (*Agropyron cristatum* L. Gaertn.), Western Wheatgrass (*Agropyron smithii* Rydb.). Examples of warm season turfgrasses are the Bermudagrasses (*Cynodon* L.C. Rich), the Zoysiagrasses (*Zoysia* Willd.), St. Augustinegrasses (*Stenotaphrum secundatum* (Walt.) Kuntze), Centipedegrass (*Eremochioa ophiuroides* (Munro.) Hack.), Carpetgrass (*Axonopus* Beauv.), Bahiagrass (*Paspalum notatum* Flugge.), Kikuyugrass (*Pennisetum clandestinum* Hochst. ex Chiov.), Buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.), Blue Grama (*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.), Sideoats Grama (*Bouteloua curtipendula* (Michx.) Torr.), and Dichondra

(*Dichondra* Forst.).

Any plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a vector of the present invention. The term "organogenesis," as used herein, means a process by which shoots and roots are developed sequentially from meristematic centers; the term "embryogenesis," as used herein, means a process by which shoots and roots develop together in a concerted fashion (not sequentially), whether from somatic cells or gametes. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, callus tissue, existing meristematic tissue (e.g., apical meristems, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem).

Plants of the present invention may take a variety of forms. The plants may be chimeras of transformed cells and non-transformed cells; the plants may be clonal transformants (e.g., all cells transformed to contain the transcription cassette); the plants may comprise grafts of transformed and untransformed tissues (e.g., a transformed root stock grafted to an untransformed scion in citrus species). The transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, first generation (or T1) transformed plants may be selfed to provide homozygous second generation (or T2) transformed plants, and the T2 plants further propagated through classical breeding techniques. A dominant selectable marker (such as nptII) can be associated with the transcription cassette to assist in breeding.

As used herein, a crop comprises a plurality of plants of the same genus or species, planted together in an agricultural field. By "agricultural field" is meant a common plot of soil or a greenhouse. Thus, the present invention provides a method of producing a crop of plants having altered metabolism of chemical compounds (such as a phenylurea herbicide), and thus having altered

resistance to the chemical compound, compared to a crop of non-transformed plants of the same genus or species, or variety.

Where a crop comprises a plurality of transgenic plants with increased resistance to phenylurea compounds according to the present invention, such compounds may be used as post-emergent herbicides to control undesirable plant species. Accordingly, a method of using phenylurea compounds as post-emergent herbicides according to the present invention comprises planting a plurality of transformed plant seed (or transformed plants) with enhanced resistance to a phenylurea herbicide, and applying that herbicide to the field after the germination and emergence of at least some of said transformed plant seed (or following the planting of transformed plants). Application of the phenylurea herbicide will selectively impact non-resistant plants.

9. Microbial decontamination:

Microbial cells useful for degrading phenylurea compounds, which cells contain and express a heterologous DNA molecule encoding a P-450 enzyme that enhances the metabolism of the phenylurea compound in the microbial cell (*e.g.*, a peptide of **SEQ ID NO:2**), are a further aspect of the present invention. Suitable host microbial cells include soil microbes (*i.e.*, those which grow in the soil) transformed to express a P-450 enzyme that enhances the metabolism of one or more phenylurea compounds by the host cell. Suitable microbes include bacteria (such as *Agrobacterium, Bacillus, Streptomyces, Nocardia,* etc.), fungi (including yeasts), and algae. Microbes can be selected, by methods known in the art of soil microbiology, to correspond to those which are typically found in the substrate to be treated. Liquids which are contaminated with phenylurea compounds may be contacted to transformed microorganisms by passing the contaminated liquid through a bioreactor which contains the microorganism. Numerous suitable bioreactor designs are known in the art. A microbial host particularly suitable for bioreactors is yeast.

Combination treatments utilizing aspects of the present invention involve

the application of a phenylurea compound in a location such as an agricultural field (*e.g.*, as a herbicide), and subsequent application of a transformed microbe as described above in an amount effective to degrade residual applied herbicide. Application of the herbicide may be carried out in accordance with known techniques.

The examples which follow are set forth to illustrate the present invention, and are not to be construed as limiting thereof.

EXAMPLE 1

Materials and Methods

a. Substrates

Phenyl-U-[^{14}C] fluometuron, phenyl-U-[^{14}C] chlortoluron, phenyl-U-[^{14}C] metolachlor, phenyl-U-[^{14}C] prosulfuron, pyrimidinyl-2- diazinon, and phenyl-U-[^{14}C] alachlor were provided by Novartis (Greensboro, North Carolina); phenyl-U-[^{14}C] bentazon was donated by BASF (Research Triangle Park, North Carolina); phenyl-U-[^{14}C] linuron, phenyl-U-[^{14}C] diuron, and carbonyl-[^{14}C] metribuzin were a gift from DuPont de Nemours (Wilmington, Delaware); carboxyl-[^{14}C] imazaquin was provided by American Cyanamid (Princeton, New Jersey).

b. Isolation of P-450 cDNAs

Random amplification of partial cDNAs encoding P-450 enzymes was conducted essentially as described by Meijer et al., *Plant Mol. Biol.* 22:379-383 (1993), using a soybean (*Glycine max* cv Dare) leaf cDNA library as the template (Dewey et al., *Plant Cell* 6:1495-1507 (1994)). Briefly, degenerate inosine-containing primers were synthesized based on the highly conserved heme-binding region. The precise sequences of these primers are described in Meijer et al. (1993). An oligo-dT primer complementary to the poly(A) tail of the cDNA clones was used in conjunction with the degenerate primers in PCR amplification assays. Amplification products were cloned into the T-tailed pCRII plasmid

(Invitrogen, San Diego, CA) and DNA sequence analysis of the first 300-400 base pairs downstream of the conserved region was used to establish whether a given amplification product represented a true P-450 cDNA.

To recover full-length versions of the partial cDNAs, a primer (5'-TGTCTAACTCCTTCCTTTTC-3') (**SEQ ID NO:19**) complementary to the pYES2 vector (the vector into which the soybean cDNA library was cloned) and a downstream primer corresponding to a segment of the 3' untranslated region for each of the unique P-450 cDNAs were used in PCR reactions using the same soybean cDNA library as the template. PCR products were again cloned into the pCRII plasmid and the entire DNA sequence was determined for the largest cDNA amplified for each unique soybean P-450.

To isolate full-length versions of the respective P-450 ORFs without including any of the 5' untranslated region (which has been shown to potentially impede gene expression in yeast (Pompon, *Eur. J. Biochem.* 177:285-293 (1988)), an additional PCR reaction was performed with two gene-specific primers. The forward primers contained a BamHI restriction site immediately followed by the ATG start codon, and the next 14-15 bases of the reading frame; the downstream primer was again specific for the 3' untranslated regions of the respective genes and included sequences specifying either EcoRI, KpnI, and SacI to facilitate subcloning of the P-450 cDNAs into the yeast expression vector, pYeDP60 (V-60; Urban et al., *Biochimie* 72:463-472 (1990)).

All PCR reactions, with the exception of the initial amplification of the partial P-450 cDNAs (see Meijer et al. (1993)), contained 0.2 ng/μl template, 2 μM of each primer, 200 μM of each dNTP, and 1.5 mM $MgCl_2$ in a final reaction volume of 50 μl. Amplification was initiated by the addition of 1.5 U EXPAND™ High Fidelity enzyme mix using conditions described by the manufacturer (Boeringer Mannheim). DNA sequence was determined by the chain termination method (Sanger et al., *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)) using fluorescent dyes (Applied Biosystems, Foster City, CA). DNA and predicted amino acid sequences were analyzed using the BLAST

algorithm and the GAP program (University of Wisconsin, Madison, Genetics Computing Group software package).

c. P-450 cDNA Expression in Yeast

Yeast transformation was performed as described by Geitz et al., *Nucleic Acids Research* 20:1425 (1992). Media composition, culturing conditions, galactose induction, and microsomal preparations were conducted according to Pompon et al., *Methods Enzymol.* 272:51-64 (1995), using a culture volume of 250 ml. Microsomal protein was quantified spectrophotometrically using the method of Waddell, *J. Lab. Clin. Med.* 48:311-314 (1956), using bovine albumin as a standard. Dithionite-reduced, carbon monoxide difference spectra was obtained as previously outlined (Estabrook and Werringloer, *Methods Enzymol.* 52:212-220 (1978)) using a Shimadzu Recording Spectrophotometer UV-240 (Shimadzu, Kyoto, Japan). P-450 protein concentrations of yeast microsomes were calculated using a millimolar extinction coefficient of 91 (Omura and Sato, *J. Biol. Chem.*, 239:2370-2378 (1964)).

d. *In vitro* Herbicide Metabolism Assays

Yeast microsomes enriched for a discrete soybean P-450 isozyme were assayed for their capacity to metabolize the ten herbicides and one insecticide listed in **Table 3**. The reaction mixtures contained 10,000 DPM (100-200 ng) radiolabeled substrate, 0.75 mM NAPDH, 2.5 mg/ml microsomal protein. Total reaction volumes were adjusted to 150 µl with 50 mM phosphate buffer (pH 7.1). The mixtures were incubated under light for 45 minutes at 27°C, arrested with 50 µl acetone and centrifuged at 14 000xg for 2 minutes. Fifty microliters of the supernatants containing radiolabeled alachlor, metolachlor, metribuzin, prosulfuron, chlortoluron, diuron, fluometuron, linuron, or diazinon were spotted onto 250 micron Whatman K6F silica plates. Radiolabeled bentazon and imazaquin-containing samples were spotted onto 200 micron Whatman LKC18F silica gel reversed-phase plates. All plates were developed in a benzene/acetone

2:1 (v/v) solvent system with the exception of prosulfuron, developed in toluene/acetone/acetic acid, 75:20:5 (v/v/v), and bentazon and imazaquin, developed in methanol/75 mM sodium acetate 40:60 (v/v). The developed plates were scanned with a Bioscan System 400 imaging scanner (Bioscan, Washington, DC), and the production of metabolites was determined based on the chromatographic profiles. For microsomes containing the expressed CYP71A10 enzyme, control experiments were also conducted to measure the NADPH-dependency, and the inhibitory effects of CO. CO treatment of the sample was achieved by gentle bubbling of the gas through the reaction mixture for 2 minutes immediately before the assay was initiated by the addition of NADPH.

e. Enzyme Kinetics

Substrate conversion was quantified by a combination of TLC analysis and scintillation spectrometry. The location of the metabolic products on the TLC plates was identified using an imaging scanner, the bands were scraped and analyzed by scintillation spectrometry. The amount of metabolite produced was calculated based on specific activity and scintillation counts. Each assay was repeated at least twice. K_m and V_{max} values were estimated using nonlinear regression analysis.

f. Mass Spectral Analysis

The reaction components used in the *in vitro* fluometuron and linuron metabolism assays were scaled up 50-fold, and the reactions were allowed to proceed for 3 hours. The substrates and the metabolites were extracted 3 times with 20 ml ethyl acetate. The extracts were combined, evaporated to dryness, and the resulting pellet was resuspended in 1 ml acetone. The samples were purified twice using preparative TLC and imaging scanning as described above . Finally, the respective bands were scraped, the compounds were eluted with acetone and flash evaporated.

Fractions of interest were analyzed by liquid chromatography/mass

spectrometry (LC/MS). Mass spectral measurements were made with a Finnigan TSQ 7000 triple quadruple mass spectrometer (QQQ) equipped with an Atmospheric Pressure Ionization (API) interface fitted with a pneumatically assisted electrospray head (Finnigan MAT, Brennan, Germany). The spray nozzle was operated at 5 kV in the positive ion mode and 4 kV in the negative ion mode. For sample introduction, the TSQ 7000 was equipped with a HPLC solvent delivery system (Perkin-Elmer 410 LC pump), a UV detector (Perkin-Elmer), a stream splitter set at 6:1 with the majority of the effluent flowing to a radioisotope flow monitor (IN/US β-RAM) and the other stream attached to the API interface. Samples were chromatographed on a reverse phase HPLC column (Inertsil 5 ODS2, 150 x 2 mm i.d.). The column was eluted at 0.4 ml/min with 95:5 of 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in methanol, respectively. Collision induced dissociation experiments (MS/MS) were conducted using argon gas with collision energy in the range of 17.5-30 eV at cell pressures of approximately 0.28 Pa. Signals were captured using a Finnigan 7000 data system.

g. NMR Analysis

Proton NMR measurements were made on a Bruker AMX-400 NMR spectrometer equipped with either a QNP or inverse probe set at 400.13 MHZ. Spectra were acquired at ambient temperature in acetonitrile-d_3. Chemical shifts were expressed as parts per million, relative to the resonance of residual acetonitrile protons at 1.93 ppm (δ).

h. Tobacco Transformation

A plant expression vector capable of mediating the constitutive expression of CYP71A10 was produced. The GUS open reading frame of the binary expression vector pBI121 (Clontech, Polo Alto, CA) was excised and replaced with the full length CYP71A10 reading frame. This placed the soybean gene under the transcriptional control of the strong constitutive CaMV 35S promoter.

The resulting construct was used to transform Agrobacterium tumefaciens strain LBA 4404 (Holsters et al., *Mol. Gen. Genetics*, 163:181-187 (1988)). Excised leaf discs of Nicotiana tabacum cv SR1 were transformed using the Agrobacterium, and kanamycin-resistant plants were selected as described by Horsch et al. *Science*, 227:1229-1231 (1985). Primary transformants were potted in a standard soil mixture, transferred to a greenhouse and their seed harvested upon maturation.

i. *In vivo* Herbicide Metabolism Assays

Seeds from primary transgenic tobacco plants transformed with CYP71A10 and control plants transformed with the pBI121 vector were grown in Petri dishes containing MS salts and 100 µg/ml kanamycin. At five weeks post-seeding, kanamycin-resistant plantlets were transplanted into pots containing soil and grown an additional two weeks. Single leaves of approximately 10 cm^2 in size were excised and their petioles inserted into 100 µl of H_2O containing radiolabeled herbicide. The leaves were placed in a growth chamber maintaining a temperature of 27°C and incubated until the entire volume of the herbicide solution was drawn up by the transpirational stream of the leaves (about 3 hrs). The leaves were subsequently transferred into an Eppendorf tube containing distilled water and further incubated for a total of 14 hours.

[^{14}C]-labeled herbicide was extracted from the leaves by grinding for 5 minutes in 250 µl methanol with a plastic pellet pestle driven by an electric drill. After centrifugation for 3 minutes at 14,000 g, 75 µl of the supernatant was spotted on a Whatman K6F silica plate and developed in a solvent system containing chloroform/ethanol/acetic acid 135:10:15 (v/v/v). The separated herbicide derivatives were visualized using an imaging scanner. Substrate conversion was quantified based on the amount of herbicide absorbed, and the ratios of the parent compound and the produced metabolites determined from the TLC profiles.

j. Herbicide Tolerance

T_1 generation seeds from CYP71A10-transformed tobacco and pBI121-transformed control plants were placed onto Petri dishes containing MS salts and linuron (using its commercial formulation LOROX 50 DF) at active ingredient concentrations ranging from 0.25 to 3.0 μM. Chlortoluron was added at 0, 1.0, 5.0 and 10.0 μM concentrations using a 99.5% pure analytical standard. The Petri dishes were incubated in a growth chamber maintaining a constant temperature of 27°C and a 16/8 hour light/dark cycle. The phytotoxic effects of the treatments were determined visually by comparison to control plants and plants grown in the absence of the herbicide. All treatments were repeated at least twice.

EXAMPLE 2

Isolation of P-450 cDNAs

To isolate cDNAs encoding P-450s from soybean, the PCR strategy described by Meijer et al. (1993) was adapted, using a soybean leaf cDNA library as the template. Degenerate, inosine-containing PCR primers were constructed corresponding to the first nine codons encoding the conserved sequence FLPFGxGxRxCxG (x = any amino acid) (**SEQ ID NO:20**), which represents an extension of the highly conserved FxxGxxxCxG motif (Bozak et al., *Proc. Natl. Acad. Sci. USA* 87:3904-3908 (1990)) (**SEQ ID NO:21**). Located near the C-terminal end of the protein, this motif defines the heme-binding region of the protein and may be regarded as a "signature" for P-450 proteins. A second nonspecific primer complementary to the poly(A) tail of the cDNA clones was used in conjunction with these degenerate primers in a PCR amplification assay. PCR amplification products were cloned into a plasmid vector and analyzed by DNA sequencing. Of 86 randomly selected individuals that were sequenced, 15 clones representing 10 unique cDNAs were identified that possessed the conserved cysteine and glycine residues of the signature

consensus (xCxG) (**SEQ ID NO:22**) immediately following the sequence defined by the degenerate PCR primers. Furthermore, homology searches of the major DNA and protein data bases revealed additional sequence identities to previously reported P-450 sequences for each of the ten unique soybean sequences (data not shown). Because this strategy only allows the recovery of sequence corresponding to the C-terminal portion of the proteins, additional PCR-based techniques were utilized to obtain cDNAs possessing the entire reading frames for each clone. Full length cDNAs were isolated for eight of the 10 individual clones and a near full length cDNA was isolated for an additional clone.

The eight full length and one near full length soybean P-450 cDNAs isolated are described in **Table 1**. The nomenclature for P-450 genes is based on amino acid sequence identity. Typically, sequences sharing >40% identity are placed in the same family, >55% identity defines members of the same subfamily, and sequences that display >97% identity are assumed to represent allelic variants, although exceptions to these designations have been noted (Nelson et al., *Pharmacogenetics*, 6:1-41 (1996)). According to this system of nomenclature, all of the nine soybean cDNAs were able to be placed within existing P-450 gene families; however, three of the sequences (CYP82C1, CYP83D1 and CYP93C1) defined new subfamilies. Although an increasing number of P-450 gene products have been assigned specific enzymatic functions (reviewed in Schuler, 1996), none of the soybean cDNAs listed in **Table 1** could be placed into families for which an *in vivo* function had been determined for any of its members.

In addition to the conserved heme-binding domain described previously, all of the predicted soybean polypeptides possess slight variations of the conserved sequence PEEFxPERF (**SEQ ID NO:23**) located approximately 30 amino acids forward of the heme-binding motif (Hallahan et al., *Biochem. Soc. Trans.* 21:1068-1073 (1993)). Also characteristic of microsomal P-450s is the presence of an N-terminal noncleavable signal sequence that serves as the membrane anchor. Immediately following this signal-anchor segment in most

microsomal P-450s is a proline-rich region that is believed to form a hinge between the catalytic cytoplasmic domain and the hydrophobic membrane anchor (Halkier, *Phytochemistry* 43:1-21 (1996)). All of the present clones (except CYP97B2) encode proteins possessing predicted signal sequences; all individuals (except CYP97B2 and CYP82C1) contain readily identifiable proline-rich domains following the signal sequence (**Table 1**). It is the identification of both of these N-terminal motifs in the CYP83D1 encoded protein (but no Met codon) that indicates that this clone is nearly full length. Interestingly, instead of possessing a predicted signal sequence and proline-rich region, the N-terminus of the polypeptide encoded by clone CYP97B2 contains a motif characteristic of a chloroplast transit peptide (data not shown).

Table 1

Soybean P-450s Isolated Using Degenerate PCR Primers

Name	GenBank Accession #	Length (amino acids)	Closest Match	Identity* %	Membrane Anchor	Proline -rich Region
CYP71A10 (SEQ ID NO:1)	AF022157	513	CYP71A1	51.7	+	+
CYP71D10 (SEQ ID NO:3)	AF022459	510	CYP71D9	50.9	+	+
CYP77A3 (SEQ ID NO:5)	AF022464	513	CYP77A1	69.8	+	+
CYP78A3 (SEQ ID NO:7)	AF022463	523	CYP78A2	53.1	+	+
CYP82C1 (SEQ ID NO:9)	AF022461	532	CYP82A3	51.1	+	–
CYP83D1** (SEQ ID NO:11)	AF022460	516	CYP71A1**	45.7	+	+
CYP93C1 (SEQ ID NO:13)	AF022462	521	CYP93B1	44.5	+	+
CYP97B2 (SEQ ID NO:15)	AF022457	576	CYP97B1	80.8	–	–
CYP98A2 (SEQ ID NO:17)	AF022458	509	CYP98A1	69.7	+	+

*Percent identity between the predicted amino acids sequences of the given soybean P-450 cDNA and the closest match identified from a BLAST search against the major gene and protein databases.

** Although this sequence shows a best match to CYP71A1, it matches poorly to some sequences of the CYP71B subfamily. As a result, the tree cluster program places it into the CYP83 family.

EXAMPLE 3

Expression of Soybean P-450 cDNAs in Yeast

Because superfluous 5' untranslated sequences from foreign genes have been shown to be capable of impeding gene expression in yeast (Pompon, 1988), an additional PCR reaction was performed on each clone that enabled the cloning of full length P-450 open reading frames (ORFs) into the yeast expression vector pYeDP60 (V-60) without including any of the endogencus 5' nontranslated flanking sequence (see Methods). For the near full length clone CYP83D1, the 5' primer was also designed to generate an "artificial" Met start codon and a Val second codon at the 5' end of the ORF. Expression in yeast of genes cloned into the V-60 vector is mediated by the strong, galactose-inducible GAL10-CYC1 promoter (Pompon et al., 1995).

Previous studies have revealed that the heterologous expression of P-450 cDNAs in yeast can be greatly enhanced in strains that have been engineered to overexpress endogenous NADPH-dependent cytochrome P-450 reductase (Pompon et al., 1995). In strain W(R), this was accomplished by exchanging the relatively weak endogenous cytochrome P-450 reductase promoter with the same GAL10-CYC1 promoter used in vector V-60 (Truan et al., *Gene* 125:49-55 (1993)). To maximize the heterologous expression of the soybean P-450 cDNAs in yeast, each of the constructs cloned into the V-60 vector was transformed into strain W(R) and microsomes were isolated from cultures that had been induced by galactose.

Reduced-CO difference spectroscopy provides a method to measure the effectiveness of expression of heterologous P-450s in yeast. Microsomal preparations corresponding to five of the soybean constructs (CYP71A10, CYP71D10, CYP77A3, CYP83D1 and CYP98A2) showed characteristic P-450 CO difference spectra with Soret peaks at 450 nm; the profile corresponding to CYP71A10 is shown in **Figure 1**. No such peaks were observed for the remaining four clones. The specific P-450 content of the five positive

microsomal preparations varied significantly, ranging from 11 pmol P-450/mg protein for construct CYP83D1 to 252 pmol P-450/mg for clone CYP77A3 as shown in **Table 2**.

Table 2

P-450 Content of Microsomes Isolated from Yeast Overexpressing Various Soybean CYPs

Clone	CYP content (pmol mg^{-1} protein)
CYP71A10	44
CYP71D10	15
CYP77A3	252
CYP83D1	11
CYP98A2	13

EXAMPLE 4

In vitro Herbicide Assays

To determine whether any of the present soybean P-450 proteins synthesized in yeast displayed significant herbicide metabolic activity, microsomal preparations possessing each of the five soybean P-450s that were effectively expressed in yeast (as judged by their reduced CO difference spectra, see above) were incubated individually with NADPH and radioisotopes of the compounds listed in **Table 3**. These substrates represent six different classes of herbicides and one organophosphate insecticide (diazinon). Upon termination of the reaction, each sample was analyzed by thin layer chromatography (TLC) to reveal potential metabolic breakdown products.

The P-450 proteins expressed from clones CYP71D10, CYP77A3, CYP83D1, and CYP98A2 displayed no apparent *in vitro* metabolic activity against any of the 11 compounds tested (data not shown). In contrast, the P-450 enzyme produced from construct CYP71A10 demonstrated considerable activity

against the phenylurea class of herbicides, but no activity against the remaining compounds. As shown in **Figure 2**, fluometuron and diuron were converted to a single metabolite; linuron and chlortoluron were transformed into two (a major and a minor) metabolites. **Figure 3** shows the chemical structures of the four phenylurea herbicides tested in this study, and the derivatives that have previously been characterized as the first metabolites produced during the detoxification of the respective herbicides in plants known to metabolize these compounds (Voss and Geissbühler, *Proc. Brit. Weed Contr. Conf.* 8:266-268 (1966); Suzuki and Casida, *J. Agric. Food Chem.* 29:1027 (1981); Ryan et al., *Pestic. Biochem. Physiol.* 16:213-221 (1981)).

To further confirm that the herbicide metabolism measured from microsomes of yeast expressing CYP71A10 was attributable to a P-450 activity, additional assays utilizing linuron as the substrate were conducted. As shown in **Figure 4**, linuron metabolizing activity is reduced approximately 37% in the presence of CO, and no metabolites are observed when NADPH is omitted from the reaction. Activity is also completely abolished upon addition of tetcyclasis, a potent P-450 inhibitor (data not shown). Furthermore, no activity is detected when microsomal preparations are used from yeast cells expressing only the V-60 control plasmid. These results verify that the observed herbicide metabolizing activity is derived from the soybean CYP71A10 cDNA.

The kinetic properties and catalytic activities of the soybean CYP71A10 protein enzyme differed significantly among the four phenylurea-type herbicide substrates. As shown in **Table 4**, turnover rates for fluometuron and linuron were considerably greater than those observed for chlortoluron and diuron. The observed reduced activity for the later two substrates is apparently not the result of decreased binding affinities since the apparent K_ms for chlortoluron and diuron are lower than those measured for fluometuron and linuron.

Table 3

Compounds Used in Metabolism Assays

Common Name	Chemical Family
Alachlor	Acetanilide
Metolachlor	Acetanilide
Bentazon	Benzothiadiazole
Imazaquin	Imidazolinone
Chlortoluron	Phenylurea
Diuron	Phenylurea
Fluometuron	Phenylurea
Linuron	Phenylurea
Prosulfuron	Sulfonylurea
Metribuzin	*as*-Triazine
Diazinon	Organophosphate

Table 4

In Vitro Kinetic Parameters of the CYP71A10 Enzyme for Four Phenylurea Substrates

Substrate	$K_{m, app}$ (μM)	V_{max} (pmol min^{-1} mg^{-1} protein)	Turnover (min^{-1})
Fluometuron	14.9 (1.0)*	303.6 (10.8)	6.8 (0.24)
Linuron	9.8 (2.1)	125.6 (12.0)	2.8 (0.27)
Chlortoluron	1.0 (0.2)	29.4 (2.2)	0.7 (0.05)
Diuron	1.5 (0.3)	16.8 (1.6)	0.4 (0.04)

* Values in parentheses represent standard error.
- Assays were repeated three times for linuron and twice for all other substrates.
- Concentration ranges (μM) used were 3.2-27.7 for fluometuron, 3.8-28.3 for linuron, 0.7-4.0 for chlortoluron, and 0.7-3.7 for diuron.

EXAMPLE 5

Analysis of Metabolites

As shown in **Figure 2**, CYP71A10-mediated degradation of phenylurea herbicides resulted in the accumulation of either one or two metabolites, depending on the particular substrate used. To determine the structure of the metabolites, the single metabolite observed in the fluometuron assay and both the major and minor metabolites generated in the linuron assay were analyzed by liquid chromatography/mass spectroscopy (LC/MS) analysis (results not shown). Analysis of the fluometuron metabolite by LC/MS in positive ion mode resulted in pseudomolecular ions at m/z 219 [$(M+H)^+$, $C_9H_9F_3N_2O$] and m/z 241 $(M+Na)^+$ that corresponds to a sodium adduct. Daughter ion spectra of m/z 219 produced a prominent m/z 162 fragment ion due to formation of the protonated trifluoromethylaniline $(C_7H_6F_3N+H)^+$. Analysis of the fluometuron metabolite by proton NMR showed a singlet at δ2.71 which integrated for 3 protons (data not shown). The NMR spectra aromatic resonances were similar to aromatic resonances observed in the parent molecule. Spectra of the fluometuron metabolite were consistent for loss of a methyl group from the parent compound.

The major linuron metabolite analyzed by LC/MS in the negative ion mode showed a pseudomolecular ion at m/z 233 $(M-H)^-$ and m/z 235 $[(M+2)-H]^-$ consistent for a molecule containing two chlorine atoms. Daughter ion spectrum at m/z 233 showed a prominent fragment ion at m/z 160 $(C_6H_4Cl_2N-H)^-$. The major linuron metabolite was 15 mass units less than parent compound which is consistent with loss of a methyl group. The position of methyl loss could not be determined based on mass spectral data alone.

The minor linuron metabolite analyzed by LC/MS gave a pseudomolecular ion at m/z 217 $(M-H)^-$ and m/z 219 $[(M+2)-H]^-$ which is consistent for a molecule containing two chlorine atoms. The daughter ion spectrum at m/z 217 showed a prominent fragment ion at m/z 160 which corresponds to formation of the dichloroaniline. The mass spectral data is consistent for the minor linuron metabolite representing N-demethoxy linuron.

These results suggest that the CYP71A10 enzyme expressed in yeast produces the same fluometuron and linuron metabolites as depicted in **Figure 3**, which shows the first metabolites produced during the detoxification of the respective herbicides in plants that are known to degrade these compounds. The metabolites of chlortoluron and diuron have not been analyzed directly, but the R_f values of the peaks observed during TLC separation are consistent with these species also representing the compounds shown in **Figure 3** (ring-hydroxymethyl chlortoluron, N-demethyl chlortoluron and N-demethyl diuron). These results indicate that the CYP71A10 enzyme functions primarily as an N-demethylase with respect to fluometuron, linuron and diuron, with some N-demethoxylase activity also observed with linuron. Using chlortoluron as a substrate, the enzyme apparently functions primarily as a methyl-ring hydroxylase and to a lesser extent as an N-demethylase.

EXAMPLE 6

Herbicide Metabolism in Transgenic Tobacco

To determine whether overexpression of the soybean CYP71A10 cDNA

in a higher plant system enhances metabolism of phenylurea herbicides, the GUS gene in the binary vector pBI121 was excised and replaced with the CYP71A10 reading frame. This construct placed the CYP71A10 cDNA under the transcriptional control of the constitutive 35S promoter of Cauliflower Mosaic Virus; kanamycin selection was facilitated via the nptII selectable marker. Agrobacterium-mediated transformation of Nicotiana tabacum cv SR1 leaf discs resulted in the recovery of several dozen independent kanamycin-resistant transformants. The plants were subsequently grown to maturity in a greenhouse and allowed to set seed.

For the herbicide metabolism assays, seeds from one randomly selected transgenic line, designated 25/2, were germinated on kanamycin-containing media to eliminate potential nontransgenic segregants. Of 17 germinated seedlings grown, only one individual was inhibited by kanamycin (data not shown). This result suggests that line 25/2 possesses more than one independently segregating transgene. Individual leaves from the 25/2 progeny were excised and incubated with radiolabeled phenylurea herbicides. As shown in **Table 5**, leaves of the kanamycin-resistant individuals of line 25/2 metabolized all of the four herbicides tested to a much greater extent than the pBI121-transformed control plants.

The relative migrations of the metabolic products revealed by TLC suggest that the products observed in the *in vivo* excised leaf assay are primarily the same as were generated from the *in vitro* assays using yeast microsomes for fluometuron, linuron and diuron (data not shown). For chlortoluron, additional metabolites were also observed. These likely represent combinations of ring-methyl hydroxylated and mono- and di-demethylated species as had been observed by Shiota et al. *Pestic. Biochem. Physiol.* 54:190-198 (1996), in their analysis of chlortoluron-resistant transgenic tobacco that overexpressed the rat CYP1A1 gene. Differences in the ratios of the observed chlortoluron metabolites were also observed between the CYP71A10-transformed and the control plants. Sixty three percent of the metabolites produced in the control leaves was N-

demethyl chlortoluron; in contrast, ring-methyl hydroxy chlortoluron was the most abundant metabolite generated in the CYP71A10-transformed leaves (47%) and only 8% of the metabolites represented N-demethyl chlortoluron.

Table 5

Phenylurea Metabolism after 14 Hours by Excised Leaves of Transgenic Tobacco Plant 25/2 Progeny

Herbicide[a]	CYP71A10-transformed	Control[b]
	% of herbicide metabolized	
Fluometuron	91 (4.5)[c]	15 (0.6)
Linuron	87 (2.0)	12 (2.6)
Chlortoluron	85 (8.1)[d]	39 (7.5)[d]
Diuron	49 (7.0)	20 (2.0)

(a) Equal amounts of herbicide (1.2 nmol) were added for each experiment.

(b) Plants transformed with the pBI121 construct were used as controls.

(c) Values in parentheses represent standard error. A single leaf was assayed from four independent 25/2 plants and three independent control plants.

(d) The major chlortoluron metabolite in the control plants represented N-demethyl chlortoluron (63%). The metabolites recovered from the CYP71A10-transformed leaves were ring-methyl hydroxy chlortoluron (47%), N-demethyl chlortoluron (8%) and other derivatives (45%).

EXAMPLE 7

Herbicide Tolerance

To establish whether enhanced herbicide metabolism leads to an increase in tolerance at the whole plant level, seeds from transgenic plant 25/2 were germinated on an agarose-base medium containing MS salts and varying

concentrations of linuron. Growth of wild-type SR1 plants and transgenic control plants expressing the GUS gene (from vector pBI121) was severely inhibited when exposed to 0.25 μM linuron and completely arrested at concentrations of 0.5 μM and higher (data not shown). As shown in **Figure 5**, progeny of plant 25/2 grown on media containing no herbicide (**Figure 5A**) appeared indistinguishable from the same seed grown in the presence of 0.5 μM linuron (**Figure 5C**), where only one of 23 germinated seedlings appeared to be inhibited by the herbicide. This ratio appears to be consistent with that observed when seeds from the same parent were grown on selective media containing kanamycin; only one of 17 seedlings failed to grow in the presence of kanamycin. **Figure 5B** shows control tobacco plants (transformed with vector pBI121), grown on media containing 0.5μM linuron. 25/2 plants tolerant to linuron levels as high as 2.5 μM linuron were observed, although an increasing percentage of the plants showed growth inhibition as the herbicide concentration was increased (**Figure 5D**). Segregation of the transgene(s) may be leading to variability in expression levels among the progeny of 25/2.

To examine whether the acquisition of herbicide tolerance is unique to line 25/2, seeds from 20 other independent CYP71A10-expressing transgenic plants were similarly germinated and grown on media containing 0.5 μM linuron. Of these, 19 lines gave rise to progeny that were linuron tolerant. The percentage of tolerant individuals for each line varied from approximately 20% to 100% (data not shown). This variation likely represents differences in the copy number, expression levels and segregation of the transgene among the independent lines.

Chlortoluron-tolerance of line 25/2 was also evident. At 1.0 μM herbicide concentration chlortoluron completely arrested the growth of the control plants (**Figure 5E**). Although growth of the 25/2 plants was modestly inhibited at this herbicide concentration, with the exception of two presumably nontransgenic segregants, the CYP71A10-transformed plants appeared healthy (**Figure 5F**). In contrast to linuron and chlortoluron, little tolerance of line 25/2

to fluometuron or diuron was observed. Herbicide concentrations that were injurious to the control plants also inhibited the growth of line 25/2 individuals. Enhanced fluometuron or diuron tolerance was only observed at the very lowest herbicide concentrations necessary to impose growth inhibition in the control plants (data not shown).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Siminszky, Balazs
Dewey, Ralph E.
Corbin, Frederick T.

(ii) TITLE OF INVENTION: Novel Cytochrome P-450 Constructs and Methods of Producing Herbicide-Resistant Transgenic Plants

(iii) NUMBER OF SEQUENCES: 23

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Bennett, Virginia C.
(B) REGISTRATION NUMBER: 37,092
(C) REFERENCE/DOCKET NUMBER: 5051-409

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1838 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 4..1542

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Lys Thr

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 513 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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    50                  55                  60

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Thr
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(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1691 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 16..1545

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Ile Tyr Phe Ile Thr Ser Ile Leu Phe Ile Phe Phe Val Phe Phe Lys
        15                  20                  25

TTA GTT CAA AGA TCG GAT TCC AAA ACC TCC TCT ACC TGC AAA TTG CCC     147
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     30                  35                  40

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 45                  50                  55                  60

GGC TCA CTG CCG GTT CAT TAC TAC TTA AAA AAT TTG GCA GAT AAG TAT     243
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                145                 150                 155

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        175                 180                 185

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Arg Asn Arg Ser Ser Glu Glu Arg Glu Ala Val Glu Asp Leu Val Asp
    270                 275                 280

GTT CTT CTC AAG TTT CAA AAG GAA TCG GAA TTT CGC TTG ACT GAT GAC      915
Val Leu Leu Lys Phe Gln Lys Glu Ser Glu Phe Arg Leu Thr Asp Asp
285                 290                 295                 300

AAC ATT AAA GCC GTC ATC CAG GAC ATA TTC ATT GGT GGA GGC GAA ACA      963
Asn Ile Lys Ala Val Ile Gln Asp Ile Phe Ile Gly Gly Gly Glu Thr
                305                 310                 315

TCA TCT TCT GTT GTG GAA TGG GGG ATG TCA GAA TTG ATA AGA AAC CCG     1011
Ser Ser Ser Val Val Glu Trp Gly Met Ser Glu Leu Ile Arg Asn Pro
            320                 325                 330
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AGG GTG ATG GAA GAA GCA CAA GCA GAG GTG AGA AGA GTG TAT GAT AGC      1059
Arg Val Met Glu Glu Ala Gln Ala Glu Val Arg Arg Val Tyr Asp Ser
        335                 340                 345

AAG GGA TAT GTG GAT GAG ACA GAA TTG CAC CAA TTG ATA TAC TTA AAG      1107
Lys Gly Tyr Val Asp Glu Thr Glu Leu His Gln Leu Ile Tyr Leu Lys
    350                 355                 360

TCC ATC ATC AAA GAA ACC ATG AGG TTA CAT CCA CCT GTG CCA TTG TTA      1155
Ser Ile Ile Lys Glu Thr Met Arg Leu His Pro Pro Val Pro Leu Leu
365                 370                 375                 380

GTT CCT AGA GTA AGT AGA GAA AGG TGC CAA ATC AAT GGA TAT GAG ATA      1203
Val Pro Arg Val Ser Arg Glu Arg Cys Gln Ile Asn Gly Tyr Glu Ile
                385                 390                 395

CCC TCT AAG ACT AGG ATC ATT ATC AAT GCT TGG GCA ATT GGA AGG AAT      1251
Pro Ser Lys Thr Arg Ile Ile Ile Asn Ala Trp Ala Ile Gly Arg Asn
            400                 405                 410

CCT AAG TAT TGG GGT GAA ACT GAG AGT TTT AAA CCT GAG AGG TTT CTT      1299
Pro Lys Tyr Trp Gly Glu Thr Glu Ser Phe Lys Pro Glu Arg Phe Leu
        415                 420                 425

AAT AGC TCC ATT GAT TTT AGG GGC ACA GAC TTT GAA TTT ATC CCA TTT      1347
Asn Ser Ser Ile Asp Phe Arg Gly Thr Asp Phe Glu Phe Ile Pro Phe
    430                 435                 440

GGT GCT GGA AGG AGG ATC TGC CCC GGC ATT ACA TTT GCC ATA CCC AAC      1395
Gly Ala Gly Arg Arg Ile Cys Pro Gly Ile Thr Phe Ala Ile Pro Asn
445                 450                 455                 460

ATT GAG TTG CCA CTT GCT CAG TTA CTT TAC CAC TTT GAT TGG AAG CTT      1443
Ile Glu Leu Pro Leu Ala Gln Leu Leu Tyr His Phe Asp Trp Lys Leu
                465                 470                 475


CCC AAT AAA ATG AAG AAT GAA GAA CTT GAC ATG ACG GAG TCA AAT GGA      1491
Pro Asn Lys Met Lys Asn Glu Glu Leu Asp Met Thr Glu Ser Asn Gly
            480                 485                 490

ATT ACT TTA CGA AGA CAA AAT GAC CTC TGC TTG ATT CCC ATT ACT CGT      1539
Ile Thr Leu Arg Arg Gln Asn Asp Leu Cys Leu Ile Pro Ile Thr Arg
        495                 500                 505

CTA CCT TAAAATGTAT GAACAATTAA TGTCATAAAC TATTTAAGTT TTATCTTTTA      1595
Leu Pro
    510

CTACTTCCAG CATTTCGTAA TTGGACAATG ACTATGATTA ACTTAAGTTA CTTCCTTATG   1655

ATTAACTTGA CATATGAATG AACATTTCTA AGATAA                             1691
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 510 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Val Met Glu Leu His Asn His Thr Pro Phe Ser Ile Tyr Phe Ile
 1               5                  10                  15

Thr Ser Ile Leu Phe Ile Phe Phe Val Phe Phe Lys Leu Val Gln Arg
            20                  25                  30

Ser Asp Ser Lys Thr Ser Ser Thr Cys Lys Leu Pro Pro Gly Pro Arg
        35                  40                  45

Thr Leu Pro Leu Ile Gly Asn Ile His Gln Ile Val Gly Ser Leu Pro
    50                  55                  60

Val His Tyr Tyr Leu Lys Asn Leu Ala Asp Lys Tyr Gly Pro Leu Met
65                  70                  75                  80

His Leu Lys Leu Gly Glu Val Ser Asn Ile Ile Val Thr Ser Pro Glu
                85                  90                  95

Met Ala Gln Glu Ile Met Lys Thr His Asp Leu Asn Phe Ser Asp Arg
            100                 105                 110

Pro Asp Phe Val Leu Ser Arg Ile Val Ser Tyr Asn Gly Ser Gly Ile
        115                 120                 125

Val Phe Ser Gln His Gly Asp Tyr Trp Arg Gln Leu Arg Lys Ile Cys
    130                 135                 140

Thr Val Glu Leu Leu Thr Ala Lys Arg Val Gln Ser Phe Arg Ser Ile
145                 150                 155                 160

Arg Glu Glu Glu Val Ala Glu Leu Val Lys Lys Ile Ala Ala Thr Ala
                165                 170                 175

Ser Glu Glu Gly Gly Ser Ile Phe Asn Leu Thr Gln Ser Ile Tyr Ser
            180                 185                 190

Met Thr Phe Gly Ile Ala Ala Arg Ala Ala Phe Gly Lys Lys Ser Arg
        195                 200                 205

Tyr Gln Gln Val Phe Ile Ser Asn Met His Lys Gln Leu Met Leu Leu
    210                 215                 220

Gly Gly Phe Ser Val Ala Asp Leu Tyr Pro Ser Ser Arg Val Phe Gln
225                 230                 235                 240

Met Met Gly Ala Thr Gly Lys Leu Glu Lys Val His Arg Val Thr Asp
                245                 250                 255

Arg Val Leu Gln Asp Ile Ile Asp Glu His Lys Asn Arg Asn Arg Ser
            260                 265                 270

Ser Glu Glu Arg Glu Ala Val Glu Asp Leu Val Asp Val Leu Leu Lys
        275                 280                 285

Phe Gln Lys Glu Ser Glu Phe Arg Leu Thr Asp Asp Asn Ile Lys Ala
```

290 295 300

Val Ile Gln Asp Ile Phe Ile Gly Gly Gly Glu Thr Ser Ser Ser Val
305 310 315 320

Val Glu Trp Gly Met Ser Glu Leu Ile Arg Asn Pro Arg Val Met Glu
325 330 335

Glu Ala Gln Ala Glu Val Arg Arg Val Tyr Asp Ser Lys Gly Tyr Val
340 345 350

Asp Glu Thr Glu Leu His Gln Leu Ile Tyr Leu Lys Ser Ile Ile Lys
355 360 365

Glu Thr Met Arg Leu His Pro Pro Val Pro Leu Leu Val Pro Arg Val
370 375 380

Ser Arg Glu Arg Cys Gln Ile Asn Gly Tyr Glu Ile Pro Ser Lys Thr
385 390 395 400

Arg Ile Ile Ile Asn Ala Trp Ala Ile Gly Arg Asn Pro Lys Tyr Trp
405 410 415

Gly Glu Thr Glu Ser Phe Lys Pro Glu Arg Phe Leu Asn Ser Ser Ile
420 425 430

Asp Phe Arg Gly Thr Asp Phe Glu Phe Ile Pro Phe Gly Ala Gly Arg
435 440 445

Arg Ile Cys Pro Gly Ile Thr Phe Ala Ile Pro Asn Ile Glu Leu Pro
450 455 460

Leu Ala Gln Leu Leu Tyr His Phe Asp Trp Lys Leu Pro Asn Lys Met
465 470 475 480

Lys Asn Glu Glu Leu Asp Met Thr Glu Ser Asn Gly Ile Thr Leu Arg
485 490 495

Arg Gln Asn Asp Leu Cys Leu Ile Pro Ile Thr Arg Leu Pro
500 505 510

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1644 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 4..1542

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAA ATG GCC ACT CTT TCC TCC TAC GAC CAC TTC ATC TTC ACT GCC TTA 48

```
    Met Ala Thr Leu Ser Ser Tyr Asp His Phe Ile Phe Thr Ala Leu
      1               5                  10                  15

GCT TTC TTC ATA TCT GGC CTA ATT TTC TTC CTC AAA CAG AAA TCC AAA        96
Ala Phe Phe Ile Ser Gly Leu Ile Phe Phe Leu Lys Gln Lys Ser Lys
                20                  25                  30

TCC AAA AAG TTC AAC CTC CCT CCA GGA CCC CCC GGG TGG CCT ATT GTT       144
Ser Lys Lys Phe Asn Leu Pro Pro Gly Pro Pro Gly Trp Pro Ile Val
            35                  40                  45

GGG AAC CTC TTC CAA GTT GCT CGT TCT GGG AAA CCT TTC TTT GAG TAT       192
Gly Asn Leu Phe Gln Val Ala Arg Ser Gly Lys Pro Phe Phe Glu Tyr
        50                  55                  60

GTG AAC GAT GTG AGA CTC AAA TAT GGC TCA ATC TTC ACC CTC AAG ATG       240
Val Asn Asp Val Arg Leu Lys Tyr Gly Ser Ile Phe Thr Leu Lys Met
    65                  70                  75

GGA ACA AGG ACC ATG ATC ATC CTC ACC GAC GCA AAA CTG GTC CAC GAG       288
Gly Thr Arg Thr Met Ile Ile Leu Thr Asp Ala Lys Leu Val His Glu
80                  85                  90                  95

GCC ATG ATC CAA AAG GGT GCA ACC TAC GCC ACC AGG CCC CCC GAG AAC       336
Ala Met Ile Gln Lys Gly Ala Thr Tyr Ala Thr Arg Pro Pro Glu Asn
                100                 105                 110


CCC ACC AGA ACC ATC TTC AGT GAA AAC AAG TTC ACC GTG AAT GCA GCG       384
Pro Thr Arg Thr Ile Phe Ser Glu Asn Lys Phe Thr Val Asn Ala Ala
            115                 120                 125

ACC TAT GGC CCC GTG TGG AAG TCG CTG AGG AGG AAC ATG GTG CAG AAC       432
Thr Tyr Gly Pro Val Trp Lys Ser Leu Arg Arg Asn Met Val Gln Asn
        130                 135                 140

ATG CTC AGC TCA ACA AGA CTT AAG GAG TTT CGC AGT GTT CGG GAC AAT       480
Met Leu Ser Ser Thr Arg Leu Lys Glu Phe Arg Ser Val Arg Asp Asn
    145                 150                 155

GCG ATG GAC AAG CTC ATC AAC AGA CTC AAG GAC GAG GCC GAG AAG AAT       528
Ala Met Asp Lys Leu Ile Asn Arg Leu Lys Asp Glu Ala Glu Lys Asn
160                 165                 170                 175

AAC GGC GTG GTT TGG GTG CTC AAG GAT GCC AGG TTT GCT GTT TTT TGC       576
Asn Gly Val Val Trp Val Leu Lys Asp Ala Arg Phe Ala Val Phe Cys
                180                 185                 190

ATA CTT GTG GCT ATG TGT TTT GGT CTT GAG ATG GAT GAG GAG ACA GTG       624
Ile Leu Val Ala Met Cys Phe Gly Leu Glu Met Asp Glu Glu Thr Val
            195                 200                 205

GAG AGA ATA GAT CAG GTT ATG AAG AGT GTT CTC ATC ACT TTG GAC CCG       672
Glu Arg Ile Asp Gln Val Met Lys Ser Val Leu Ile Thr Leu Asp Pro
        210                 215                 220

AGA ATT GAT GAC TAT CTT CCA ATT CTA AGC CCC TTT TTC TCA AAG CAA       720
Arg Ile Asp Asp Tyr Leu Pro Ile Leu Ser Pro Phe Phe Ser Lys Gln
    225                 230                 235
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AGA AAG AAA GCC TTG GAG GTT CGC AGA GAA CAG GTT GAG TTC TTA GTT      768
Arg Lys Lys Ala Leu Glu Val Arg Arg Glu Gln Val Glu Phe Leu Val
240                 245                 250                 255

CCA ATT ATA GAA CAA AGA AGA AGA GCA ATT CAA AAC CCT GGG TCA GAT      816
Pro Ile Ile Glu Gln Arg Arg Arg Ala Ile Gln Asn Pro Gly Ser Asp
                260                 265                 270

CAC ACC GCC ACA ACG TTT TCC TAC CTA GAC ACA CTT TTT GAC CTC AAA      864
His Thr Ala Thr Thr Phe Ser Tyr Leu Asp Thr Leu Phe Asp Leu Lys
            275                 280                 285

GTT GAA GGG AAG AAA TCA GCA CCC TCT GAT GCA GAA TTG GTG TCT TTA      912
Val Glu Gly Lys Lys Ser Ala Pro Ser Asp Ala Glu Leu Val Ser Leu
        290                 295                 300

TGC TCA GAG TTT CTT AAC GGT GGC ACA GAC ACA ACA GCA ACA GCG GTT      960
Cys Ser Glu Phe Leu Asn Gly Gly Thr Asp Thr Thr Ala Thr Ala Val
    305                 310                 315

GAG TGG GGC ATA GCA CAG CTC ATA GCG AAC CCT AAC GTT CAG ACA AAG     1008
Glu Trp Gly Ile Ala Gln Leu Ile Ala Asn Pro Asn Val Gln Thr Lys
320                 325                 330                 335

CTG TAC GAG GAA ATA AAG AGA ACG GTG GGA GAG AAG AAG GTG GAT GAA     1056
Leu Tyr Glu Glu Ile Lys Arg Thr Val Gly Glu Lys Lys Val Asp Glu
                340                 345                 350

AAG GAC GTT GAG AAA ATG CCA TAC CTA CAC GCT GTG GTG AAG GAG CTT     1104
Lys Asp Val Glu Lys Met Pro Tyr Leu His Ala Val Val Lys Glu Leu
            355                 360                 365

CTA AGA AAG CAC CCT CCA ACA CAC TTT GTG CTA ACA CAT GCT GTG ACT     1152
Leu Arg Lys His Pro Pro Thr His Phe Val Leu Thr His Ala Val Thr
        370                 375                 380

GAG CCC ACC ACT TTG GGA GGG TAT GAC ATA CCA ATT GAT GCA AAT GTT     1200
Glu Pro Thr Thr Leu Gly Gly Tyr Asp Ile Pro Ile Asp Ala Asn Val
    385                 390                 395

GAG GTG TAC ACA CCA GCC ATT GCT GAG GAC CCC AAA AAT TGG TTA AAC     1248
Glu Val Tyr Thr Pro Ala Ile Ala Glu Asp Pro Lys Asn Trp Leu Asn
400                 405                 410                 415

CCT GAG AAG TTT GAC CCT GAG AGA TTC ATC TCT GGG GGT GAG GAA GCA     1296
Pro Glu Lys Phe Asp Pro Glu Arg Phe Ile Ser Gly Gly Glu Glu Ala
                420                 425                 430

GAC ATA ACT GGG GTC ACA GGG GTG AAG ATG ATG CCA TTT GGG GTT GGG     1344
Asp Ile Thr Gly Val Thr Gly Val Lys Met Met Pro Phe Gly Val Gly
            435                 440                 445

AGA AGG ATT TGC CCT GGC TTG GCT ATG GCC ACA GTG CAT ATT CAC CTC     1392
Arg Arg Ile Cys Pro Gly Leu Ala Met Ala Thr Val His Ile His Leu
        450                 455                 460

ATG ATG GCA AGG ATG GTG CAG GAG TTT GAG TGG GGT GCA TAC CCT CCA     1440
Met Met Ala Arg Met Val Gln Glu Phe Glu Trp Gly Ala Tyr Pro Pro
    465                 470                 475
```

```
GAG AAG AAG ATG GAT TTC ACT GGC AAG TGG GAG TTC ACT GTG GTC ATG      1488
Glu Lys Lys Met Asp Phe Thr Gly Lys Trp Glu Phe Thr Val Val Met
480                 485                 490                 495

AAG GAG TCT CTA AGA GCA ACC ATC AAA CCA AGA GGA GGA GAA AAA GTG      1536
Lys Glu Ser Leu Arg Ala Thr Ile Lys Pro Arg Gly Gly Glu Lys Val
                500                 505                 510

AAG TTG TAAAATTTTC CTGCTTCTAT TCTTCTGGGT TTTAAATTTC ACAGACAACA      1592
Lys Leu

TAAATATTAT TGCTATTATC ATCATCATAT ATGTATACAT CATCATGGTT AC            1644
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 513 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Thr Leu Ser Ser Tyr Asp His Phe Ile Phe Thr Ala Leu Ala
 1               5                  10                  15

Phe Phe Ile Ser Gly Leu Ile Phe Phe Leu Lys Gln Lys Ser Lys Ser
            20                  25                  30

Lys Lys Phe Asn Leu Pro Pro Gly Pro Pro Gly Trp Pro Ile Val Gly
        35                  40                  45

Asn Leu Phe Gln Val Ala Arg Ser Gly Lys Pro Phe Phe Glu Tyr Val
    50                  55                  60

Asn Asp Val Arg Leu Lys Tyr Gly Ser Ile Phe Thr Leu Lys Met Gly
65                  70                  75                  80

Thr Arg Thr Met Ile Ile Leu Thr Asp Ala Lys Leu Val His Glu Ala
                85                  90                  95

Met Ile Gln Lys Gly Ala Thr Tyr Ala Thr Arg Pro Pro Glu Asn Pro
            100                 105                 110

Thr Arg Thr Ile Phe Ser Glu Asn Lys Phe Thr Val Asn Ala Ala Thr
        115                 120                 125

Tyr Gly Pro Val Trp Lys Ser Leu Arg Arg Asn Met Val Gln Asn Met
    130                 135                 140

Leu Ser Ser Thr Arg Leu Lys Glu Phe Arg Ser Val Arg Asp Asn Ala
145                 150                 155                 160

Met Asp Lys Leu Ile Asn Arg Leu Lys Asp Glu Ala Glu Lys Asn Asn
                165                 170                 175
```

Gly Val Val Trp Val Leu Lys Asp Ala Arg Phe Ala Val Phe Cys Ile
180 185 190

Leu Val Ala Met Cys Phe Gly Leu Glu Met Asp Glu Glu Thr Val Glu
195 200 205

Arg Ile Asp Gln Val Met Lys Ser Val Leu Ile Thr Leu Asp Pro Arg
210 215 220

Ile Asp Asp Tyr Leu Pro Ile Leu Ser Pro Phe Phe Ser Lys Gln Arg
225 230 235 240

Lys Lys Ala Leu Glu Val Arg Arg Glu Gln Val Glu Phe Leu Val Pro
245 250 255

Ile Ile Glu Gln Arg Arg Arg Ala Ile Gln Asn Pro Gly Ser Asp His
260 265 270

Thr Ala Thr Thr Phe Ser Tyr Leu Asp Thr Leu Phe Asp Leu Lys Val
275 280 285

Glu Gly Lys Lys Ser Ala Pro Ser Asp Ala Glu Leu Val Ser Leu Cys
290 295 300

Ser Glu Phe Leu Asn Gly Gly Thr Asp Thr Thr Ala Thr Ala Val Glu
305 310 315 320

Trp Gly Ile Ala Gln Leu Ile Ala Asn Pro Asn Val Gln Thr Lys Leu
325 330 335

Tyr Glu Glu Ile Lys Arg Thr Val Gly Glu Lys Lys Val Asp Glu Lys
340 345 350

Asp Val Glu Lys Met Pro Tyr Leu His Ala Val Val Lys Glu Leu Leu
355 360 365

Arg Lys His Pro Pro Thr His Phe Val Leu Thr His Ala Val Thr Glu
370 375 380

Pro Thr Thr Leu Gly Gly Tyr Asp Ile Pro Ile Asp Ala Asn Val Glu
385 390 395 400

Val Tyr Thr Pro Ala Ile Ala Glu Asp Pro Lys Asn Trp Leu Asn Pro
405 410 415

Glu Lys Phe Asp Pro Glu Arg Phe Ile Ser Gly Gly Glu Glu Ala Asp
420 425 430

Ile Thr Gly Val Thr Gly Val Lys Met Met Pro Phe Gly Val Gly Arg
435 440 445

Arg Ile Cys Pro Gly Leu Ala Met Ala Thr Val His Ile His Leu Met
450 455 460

Met Ala Arg Met Val Gln Glu Phe Glu Trp Gly Ala Tyr Pro Pro Glu
465 470 475 480

Lys Lys Met Asp Phe Thr Gly Lys Trp Glu Phe Thr Val Val Met Lys
485 490 495

```
Glu Ser Leu Arg Ala Thr Ile Lys Pro Arg Gly Gly Glu Lys Val Lys
            500                 505                 510

Leu
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1611 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 20..1588

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
AAGCACTATC CCTCCCACC ATG ACA AGC CAC ATT GAC GAC AAC CTC TGG ATA      52
                     Met Thr Ser His Ile Asp Asp Asn Leu Trp Ile
                       1               5                  10

ATA GCC CTG ACC TCG AAA TGC ACC CAA GAA AAC CTT GCA TGG GTC CTT     100
Ile Ala Leu Thr Ser Lys Cys Thr Gln Glu Asn Leu Ala Trp Val Leu
             15                  20                  25

TTG ATC ATG GGC TCA CTC TGG TTA ACC ATG ACT TTC TAT TAC TGG TCA     148
Leu Ile Met Gly Ser Leu Trp Leu Thr Met Thr Phe Tyr Tyr Trp Ser
         30                  35                  40

CAC CCC GGT GGT CCT GCC TGG GGC AAG TAC TAC ACC TAC TCT CCC CCC     196
His Pro Gly Gly Pro Ala Trp Gly Lys Tyr Tyr Thr Tyr Ser Pro Pro
     45                  50                  55

CTT TCA ATC ATT CCC GGT CCC AAA GGC TTC CCT CTT ATT GGA AGC ATG     244
Leu Ser Ile Ile Pro Gly Pro Lys Gly Phe Pro Leu Ile Gly Ser Met
 60                  65                  70                  75

GGC CTC ATG ACT TCC CTG GCC CAT CAC CGT ATC GCA GCC GCG GCC GCC     292
Gly Leu Met Thr Ser Leu Ala His His Arg Ile Ala Ala Ala Ala Ala
                 80                  85                  90

ACA TGC AGA GCC AAG CGC CTC ATG GCC TTT AGT CTC GGC GAC ACA CGT     340
Thr Cys Arg Ala Lys Arg Leu Met Ala Phe Ser Leu Gly Asp Thr Arg
             95                  100                 105

GTC ATC GTC ACG TGC CAC CCC GAC GTG GCC AAG GAG ATT CTC AAC AGC     388
Val Ile Val Thr Cys His Pro Asp Val Ala Lys Glu Ile Leu Asn Ser
         110                 115                 120

TCC GTC TTC GCC GAT CGT CCC GTC AAA GAA TCC GCA TAC AGC CTC ATG     436
Ser Val Phe Ala Asp Arg Pro Val Lys Glu Ser Ala Tyr Ser Leu Met
     125                 130                 135
```

```
TTT AAC CGC GCC ATC GGC TTC GCC TCT TAC GGA GTT TAC TGG CGA AGC      484
Phe Asn Arg Ala Ile Gly Phe Ala Ser Tyr Gly Val Tyr Trp Arg Ser
140                 145                 150                 155

CTC AGG AGA ATC GCC TCT AAT CAC CTC TTC TGC CCC CGC CAG ATA AAA      532
Leu Arg Arg Ile Ala Ser Asn His Leu Phe Cys Pro Arg Gln Ile Lys
                160                 165                 170

GCC TCT GAG CTC CAA CGC TCT CAA ATC GCC GCC CAA ATG GTT CAC ATC      580
Ala Ser Glu Leu Gln Arg Ser Gln Ile Ala Ala Gln Met Val His Ile
            175                 180                 185

CTA AAT AAC AAG CGC CAC CGC AGC TTA CGT GTT CGC CAA GTG CTG AAA      628
Leu Asn Asn Lys Arg His Arg Ser Leu Arg Val Arg Gln Val Leu Lys
        190                 195                 200

AAG GCT TCG CTC AGT AAC ATG ATG TGC TCC GTG TTT GGA CAA GAG TAT      676
Lys Ala Ser Leu Ser Asn Met Met Cys Ser Val Phe Gly Gln Glu Tyr
    205                 210                 215

AAG CTG CAC GAC CCA AAC AGC GGA ATG GAA GAC CTT GGA ATA TTA GTG      724
Lys Leu His Asp Pro Asn Ser Gly Met Glu Asp Leu Gly Ile Leu Val
220                 225                 230                 235

GAC CAA GGT TAT GAC CTG TTG GGC CTG TTT AAT TGG GCC GAC CAC CTT      772
Asp Gln Gly Tyr Asp Leu Leu Gly Leu Phe Asn Trp Ala Asp His Leu
                240                 245                 250

CCT TTT CTT GCA CAT TTC GAC GCC CAA AAT ATC CGG TTC AGG TGC TCC      820
Pro Phe Leu Ala His Phe Asp Ala Gln Asn Ile Arg Phe Arg Cys Ser
            255                 260                 265

AAC CTC GTC CCC ATG GTG AAC CGT TTC GTC GGC ACA ATC ATC GCT GAA      868
Asn Leu Val Pro Met Val Asn Arg Phe Val Gly Thr Ile Ile Ala Glu
        270                 275                 280

CAC CGA GCT AGT AAA ACC GAA ACC AAT CGT GAT TTT GTT GAC GTC TTG      916
His Arg Ala Ser Lys Thr Glu Thr Asn Arg Asp Phe Val Asp Val Leu
    285                 290                 295

CTC TCT CTC CCG GAA CCT GAT CAA TTA TCA GAC TCC GAC ATG ATC GCT      964
Leu Ser Leu Pro Glu Pro Asp Gln Leu Ser Asp Ser Asp Met Ile Ala
300                 305                 310                 315

GTA CTT TGG GAA ATG ATA TTC AGA GGA ACG GAC ACG GTA GCG GTT TTG     1012
Val Leu Trp Glu Met Ile Phe Arg Gly Thr Asp Thr Val Ala Val Leu
                320                 325                 330

ATA GAG TGG ATA CTC GCG AGG ATG GCG CTT CAT CCT CAT GTG CAG TCC     1060
Ile Glu Trp Ile Leu Ala Arg Met Ala Leu His Pro His Val Gln Ser
            335                 340                 345

AAA GTT CAA GAG GAG CTA GAT GCA GTT GTC GGA AAA GCA CGC GCC GTC     1108
Lys Val Gln Glu Glu Leu Asp Ala Val Val Gly Lys Ala Arg Ala Val
        350                 355                 360

GCA GAG GAT GAC GTG GCA GTG ATG ACG TAC CTA CCA GCG GTG GTG AAG     1156
Ala Glu Asp Asp Val Ala Val Met Thr Tyr Leu Pro Ala Val Val Lys
    365                 370                 375
```

```
GAG GTG CTG CGG CTG CAC CCG CCG GGC CCA CTT CTA TCA TGG GCC CGC      1204
Glu Val Leu Arg Leu His Pro Pro Gly Pro Leu Leu Ser Trp Ala Arg
380                 385                 390                 395

TTG TCC ATC AAT GAT ACG ACC ATT GAT GGG TAT CAC GTA CCT GCG GGG      1252
Leu Ser Ile Asn Asp Thr Thr Ile Asp Gly Tyr His Val Pro Ala Gly
                400                 405                 410


ACC ACT GCT ATG GTC AAC ACG TGG GCT ATT TGC AGG GAC CCA CAC GTG      1300
Thr Thr Ala Met Val Asn Thr Trp Ala Ile Cys Arg Asp Pro His Val
            415                 420                 425

TGG AAG GAC CCA CTC GAA TTT ATG CCC GAG AGG TTT GTC ACT GCG GGT      1348
Trp Lys Asp Pro Leu Glu Phe Met Pro Glu Arg Phe Val Thr Ala Gly
        430                 435                 440

GGA GAT GCC GAA TTT TCG ATA CTC GGG TCG GAT CCA AGA CTT GCT CCA      1396
Gly Asp Ala Glu Phe Ser Ile Leu Gly Ser Asp Pro Arg Leu Ala Pro
    445                 450                 455

TTT GGG TCG GGT AGG AGA GCG TGC CCA GGG AAG ACT CTT GGA TGG GCT      1444
Phe Gly Ser Gly Arg Arg Ala Cys Pro Gly Lys Thr Leu Gly Trp Ala
460                 465                 470                 475

ACG GTG AAC TTT TGG GTG GCG TCG CTC TTG CAT GAG TTC GAA TGG GTA      1492
Thr Val Asn Phe Trp Val Ala Ser Leu Leu His Glu Phe Glu Trp Val
                480                 485                 490

CCG TCT GAT GAG AAG GGT GTT GAT CTG ACG GAG GTG CTG AAG CTC TCT      1540
Pro Ser Asp Glu Lys Gly Val Asp Leu Thr Glu Val Leu Lys Leu Ser
            495                 500                 505

AGT GAA ATG GCT AAC CCT CTC ACC GTC AAA GTG CGC CCC AGG CGT GGA      1588
Ser Glu Met Ala Asn Pro Leu Thr Val Lys Val Arg Pro Arg Arg Gly
        510                 515                 520

TAAGAGAGAG TTGAAGCTTT TAT                                            1611
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 523 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Thr Ser His Ile Asp Asp Asn Leu Trp Ile Ile Ala Leu Thr Ser
  1               5                  10                  15

Lys Cys Thr Gln Glu Asn Leu Ala Trp Val Leu Leu Ile Met Gly Ser
             20                  25                  30

Leu Trp Leu Thr Met Thr Phe Tyr Tyr Trp Ser His Pro Gly Gly Pro
         35                  40                  45
```

Ala Trp Gly Lys Tyr Tyr Thr Tyr Ser Pro Pro Leu Ser Ile Ile Pro
50 55 60

Gly Pro Lys Gly Phe Pro Leu Ile Gly Ser Met Gly Leu Met Thr Ser
65 70 75 80

Leu Ala His His Arg Ile Ala Ala Ala Ala Ala Thr Cys Arg Ala Lys
85 90 95

Arg Leu Met Ala Phe Ser Leu Gly Asp Thr Arg Val Ile Val Thr Cys
100 105 110

His Pro Asp Val Ala Lys Glu Ile Leu Asn Ser Ser Val Phe Ala Asp
115 120 125

Arg Pro Val Lys Glu Ser Ala Tyr Ser Leu Met Phe Asn Arg Ala Ile
130 135 140

Gly Phe Ala Ser Tyr Gly Val Tyr Trp Arg Ser Leu Arg Arg Ile Ala
145 150 155 160

Ser Asn His Leu Phe Cys Pro Arg Gln Ile Lys Ala Ser Glu Leu Gln
165 170 175

Arg Ser Gln Ile Ala Ala Gln Met Val His Ile Leu Asn Asn Lys Arg
180 185 190

His Arg Ser Leu Arg Val Arg Gln Val Leu Lys Lys Ala Ser Leu Ser
195 200 205

Asn Met Met Cys Ser Val Phe Gly Gln Glu Tyr Lys Leu His Asp Pro
210 215 220

Asn Ser Gly Met Glu Asp Leu Gly Ile Leu Val Asp Gln Gly Tyr Asp
225 230 235 240

Leu Leu Gly Leu Phe Asn Trp Ala Asp His Leu Pro Phe Leu Ala His
245 250 255

Phe Asp Ala Gln Asn Ile Arg Phe Arg Cys Ser Asn Leu Val Pro Met
260 265 270

Val Asn Arg Phe Val Gly Thr Ile Ile Ala Glu His Arg Ala Ser Lys
275 280 285

Thr Glu Thr Asn Arg Asp Phe Val Asp Val Leu Leu Ser Leu Pro Glu
290 295 300

Pro Asp Gln Leu Ser Asp Ser Asp Met Ile Ala Val Leu Trp Glu Met
305 310 315 320

Ile Phe Arg Gly Thr Asp Thr Val Ala Val Leu Ile Glu Trp Ile Leu
325 330 335

Ala Arg Met Ala Leu His Pro His Val Gln Ser Lys Val Gln Glu Glu
340 345 350

Leu Asp Ala Val Val Gly Lys Ala Arg Ala Val Ala Glu Asp Asp Val
355 360 365

```
Ala Val Met Thr Tyr Leu Pro Ala Val Val Lys Glu Val Leu Arg Leu
    370                 375                 380

His Pro Pro Gly Pro Leu Leu Ser Trp Ala Arg Leu Ser Ile Asn Asp
385                 390                 395                 400

Thr Thr Ile Asp Gly Tyr His Val Pro Ala Gly Thr Thr Ala Met Val
                405                 410                 415

Asn Thr Trp Ala Ile Cys Arg Asp Pro His Val Trp Lys Asp Pro Leu
            420                 425                 430

Glu Phe Met Pro Glu Arg Phe Val Thr Ala Gly Gly Asp Ala Glu Phe
        435                 440                 445

Ser Ile Leu Gly Ser Asp Pro Arg Leu Ala Pro Phe Gly Ser Gly Arg
    450                 455                 460

Arg Ala Cys Pro Gly Lys Thr Leu Gly Trp Ala Thr Val Asn Phe Trp
465                 470                 475                 480

Val Ala Ser Leu Leu His Glu Phe Glu Trp Val Pro Ser Asp Glu Lys
                485                 490                 495

Gly Val Asp Leu Thr Glu Val Leu Lys Leu Ser Ser Glu Met Ala Asn
            500                 505                 510

Pro Leu Thr Val Lys Val Arg Pro Arg Arg Gly
        515                 520
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1788 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 6..1601

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GGGTC ATG GGC ATG GCC ATG GAT GCT TTC CAG CAC CAA ACT CTC ATT          47
      Met Gly Met Ala Met Asp Ala Phe Gln His Gln Thr Leu Ile
        1               5                   10

TCC ATC ATT CTG GCC ATG TTA GTA GGC GTG TTG ATT TAT GGC TTA AAG        95
Ser Ile Ile Leu Ala Met Leu Val Gly Val Leu Ile Tyr Gly Leu Lys
 15                  20                  25                  30

AGA ACA CAT AGT GGC CAT GGC AAG ATC TGT AGT GCA CCT CAA GCA GGA       143
Arg Thr His Ser Gly His Gly Lys Ile Cys Ser Ala Pro Gln Ala Gly
                35                  40                  45
```

```
GGA GCA TGG CCA ATT ATT GGC CAT TTA CAC CTC TTT GGG GGT CAT CAA       191
Gly Ala Trp Pro Ile Ile Gly His Leu His Leu Phe Gly Gly His Gln
               50                  55                  60

CAT ACT CAC AAA ACA CTT GGG ATA ATG GCA GAG AAA CAT GGA CCA ATT       239
His Thr His Lys Thr Leu Gly Ile Met Ala Glu Lys His Gly Pro Ile
          65                  70                  75

TTC ACA ATA AAG CTT GGT TCA TAC AAA GTT CTT GTA TTG AGT AGC TGG       287
Phe Thr Ile Lys Leu Gly Ser Tyr Lys Val Leu Val Leu Ser Ser Trp
      80                  85                  90

GAG ATG GCC AAG GAG TGT TTC ACT GTC CAT GAC AAA GCA TTT TCT ACC       335
Glu Met Ala Lys Glu Cys Phe Thr Val His Asp Lys Ala Phe Ser Thr
 95                 100                 105                 110

AGA CCC TGT GTT GCA GCC TCA AAG CTA ATG GGC TAC AAC TAT GCC ATG       383
Arg Pro Cys Val Ala Ala Ser Lys Leu Met Gly Tyr Asn Tyr Ala Met
                115                 120                 125

TTT GGC TTC ACT CCT TAT GGT CCT TAT TGG CGT GAG ATA AGG AAA TTA       431
Phe Gly Phe Thr Pro Tyr Gly Pro Tyr Trp Arg Glu Ile Arg Lys Leu
            130                 135                 140

ACT ACT ATT CAG CTT CTA TCT AAC CAC CGG CTT GAA CTG CTG AAG AAC       479
Thr Thr Ile Gln Leu Leu Ser Asn His Arg Leu Glu Leu Leu Lys Asn
        145                 150                 155

ACA AGA ACA TCT GAG TCA GAA GTT GCA ATA AGA GAG CTT TAT AAG TTG       527
Thr Arg Thr Ser Glu Ser Glu Val Ala Ile Arg Glu Leu Tyr Lys Leu
    160                 165                 170

TGG TCT AGA GAA GGT TGT CCA AAG GGA GGG GTT TTG GTA GAT ATG AAG       575
Trp Ser Arg Glu Gly Cys Pro Lys Gly Gly Val Leu Val Asp Met Lys
175                 180                 185                 190

CAG TGG TTT GGG GAT TTA ACT CAT AAT ATT GTT CTG AGA ATG GTG AGA       623
Gln Trp Phe Gly Asp Leu Thr His Asn Ile Val Leu Arg Met Val Arg
                195                 200                 205

GGG AAG CCA TAC TAT GAT GGT GCT AGT GAT GAT TAT GCA GAA GGT GAA       671
Gly Lys Pro Tyr Tyr Asp Gly Ala Ser Asp Asp Tyr Ala Glu Gly Glu
            210                 215                 220

GCA AGA AGG TAC AAG AAA GTT ATG GGA GAG TGT GTG AGT TTG TTT GGG       719
Ala Arg Arg Tyr Lys Lys Val Met Gly Glu Cys Val Ser Leu Phe Gly
        225                 230                 235

GTG TTT GTG TTA TCT GAT GCT ATT CCA TTT CTG GGG TGG TTG GAC ATC       767
Val Phe Val Leu Ser Asp Ala Ile Pro Phe Leu Gly Trp Leu Asp Ile
    240                 245                 250

AAC GGA TAT GAA AAG GCC ATG AAG AGA ACT GCA AGT GAA TTG GAT CCT       815
Asn Gly Tyr Glu Lys Ala Met Lys Arg Thr Ala Ser Glu Leu Asp Pro
255                 260                 265                 270

CTG GTT GAA GGG TGG TTA GAG GAA CAC AAA AGG AAA AGA GCT TTC AAT       863
Leu Val Glu Gly Trp Leu Glu Glu His Lys Arg Lys Arg Ala Phe Asn
```

```
                  275                 280                 285

ATG GAT GCA AAA GAA GAA CAG GAT AAT TTC ATG GAT GTC ATG CTG AAT      911
Met Asp Ala Lys Glu Glu Gln Asp Asn Phe Met Asp Val Met Leu Asn
            290                 295                 300

GTT CTG AAA GAT GCA GAG ATT TCT GGT TAT GAT TCA GAT ACC ATC ATC      959
Val Leu Lys Asp Ala Glu Ile Ser Gly Tyr Asp Ser Asp Thr Ile Ile
        305                 310                 315

AAG GCT ACT TGT CTG AAT CTG ATT TTA GCA GGA AGC GAC ACC ACC ATG     1007
Lys Ala Thr Cys Leu Asn Leu Ile Leu Ala Gly Ser Asp Thr Thr Met
    320                 325                 330

ATT TCA CTA ACA TGG GTG CTA TCT CTG CTA CTT AAC CAT CAA ATG GAA     1055
Ile Ser Leu Thr Trp Val Leu Ser Leu Leu Leu Asn His Gln Met Glu
335                 340                 345                 350

CTA AAA AAA GTC CAA GAT GAA TTG GAC ACT TAT ATT GGG AAG GAC AGG     1103
Leu Lys Lys Val Gln Asp Glu Leu Asp Thr Tyr Ile Gly Lys Asp Arg
                355                 360                 365

AAG GTG GAA GAA TCT GAC ATA ACC AAG TTG GTG TAC CTC CAA GCC ATT     1151
Lys Val Glu Glu Ser Asp Ile Thr Lys Leu Val Tyr Leu Gln Ala Ile
            370                 375                 380

GTG AAG GAA ACA ATG CGG CTG TAT CCA CCA AGT CCT CTT ATC ACC CTT     1199
Val Lys Glu Thr Met Arg Leu Tyr Pro Pro Ser Pro Leu Ile Thr Leu
        385                 390                 395

CGT GCA GCC ATG GAA GAC TGC ACC TTC TCA GGT GGC TAT CAC ATT CCT     1247
Arg Ala Ala Met Glu Asp Cys Thr Phe Ser Gly Gly Tyr His Ile Pro
    400                 405                 410

GCT GGG ACA CGT TTA ATG GTG AAT GCT TGG AAG ATC CAC CGG GAT GGT     1295
Ala Gly Thr Arg Leu Met Val Asn Ala Trp Lys Ile His Arg Asp Gly
415                 420                 425                 430

CGT GTT TGG AGT GAT CCT CAT GAT TTC AAG CCT GGA AGG TTC TTG ACA     1343
Arg Val Trp Ser Asp Pro His Asp Phe Lys Pro Gly Arg Phe Leu Thr
                435                 440                 445

AGC CAC AAA GAT GTT GAT GTG AAG GGT CAG AAC TAT GAG CTC GTC CCT     1391
Ser His Lys Asp Val Asp Val Lys Gly Gln Asn Tyr Glu Leu Val Pro
            450                 455                 460

TTT GGT TCT GGA AGG AGA GCA TGC CCT GGA GCC TCG CTG GCT CTG CGT     1439
Phe Gly Ser Gly Arg Arg Ala Cys Pro Gly Ala Ser Leu Ala Leu Arg
        465                 470                 475

GTG GTG CAC TTG ACC ATG GCT AGA CTG TTA CAT TCT TTC AAT GTT GCT     1487
Val Val His Leu Thr Met Ala Arg Leu Leu His Ser Phe Asn Val Ala
    480                 485                 490

TCT CCT TCA AAT CAA GTT GTG GAC ATG ACA GAG AGC ATT GGA CTC ACA     1535
Ser Pro Ser Asn Gln Val Val Asp Met Thr Glu Ser Ile Gly Leu Thr
495                 500                 505                 510

AAT TTA AAA GCA ACC CCG CTT GAA ATT CTC CTA ACT CCA CGT CTA GAC     1583
```

```
Asn Leu Lys Ala Thr Pro Leu Glu Ile Leu Leu Thr Pro Arg Leu Asp
                515                 520                 525

ACC AAA CTT TAT GAG AAC TAGATTAAAT TAAGCTAGTT TTCTCCCAAA          1631
Thr Lys Leu Tyr Glu Asn
            530

TAAGGGGAGG GGTCCTCTAG GTCCTGAAAT CGGGTAATAA CAATAACATG GTTAATCCAG  1691

CTTCCATGTA GGATAATGAT TATTCACTCA TGGGTCACCT TTTAATGGAG CCTCAGTGTA  1751

TTATAATAAC TCCAAACTTG TGGGTCACAA TCCCCCC                           1788
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 532 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Gly Met Ala Met Asp Ala Phe Gln His Gln Thr Leu Ile Ser Ile
 1               5                  10                  15

Ile Leu Ala Met Leu Val Gly Val Leu Ile Tyr Gly Leu Lys Arg Thr
            20                  25                  30

His Ser Gly His Gly Lys Ile Cys Ser Ala Pro Gln Ala Gly Gly Ala
        35                  40                  45

Trp Pro Ile Ile Gly His Leu His Leu Phe Gly Gly His Gln His Thr
    50                  55                  60

His Lys Thr Leu Gly Ile Met Ala Glu Lys His Gly Pro Ile Phe Thr
65                  70                  75                  80

Ile Lys Leu Gly Ser Tyr Lys Val Leu Val Leu Ser Ser Trp Glu Met
                85                  90                  95

Ala Lys Glu Cys Phe Thr Val His Asp Lys Ala Phe Ser Thr Arg Pro
            100                 105                 110

Cys Val Ala Ala Ser Lys Leu Met Gly Tyr Asn Tyr Ala Met Phe Gly
        115                 120                 125

Phe Thr Pro Tyr Gly Pro Tyr Trp Arg Glu Ile Arg Lys Leu Thr Thr
    130                 135                 140

Ile Gln Leu Leu Ser Asn His Arg Leu Glu Leu Leu Lys Asn Thr Arg
145                 150                 155                 160

Thr Ser Glu Ser Glu Val Ala Ile Arg Glu Leu Tyr Lys Leu Trp Ser
                165                 170                 175

Arg Glu Gly Cys Pro Lys Gly Gly Val Leu Val Asp Met Lys Gln Trp
            180                 185                 190
```

Phe Gly Asp Leu Thr His Asn Ile Val Leu Arg Met Val Arg Gly Lys
195 200 205

Pro Tyr Tyr Asp Gly Ala Ser Asp Asp Tyr Ala Glu Gly Glu Ala Arg
210 215 220

Arg Tyr Lys Lys Val Met Gly Glu Cys Val Ser Leu Phe Gly Val Phe
225 230 235 240

Val Leu Ser Asp Ala Ile Pro Phe Leu Gly Trp Leu Asp Ile Asn Gly
245 250 255

Tyr Glu Lys Ala Met Lys Arg Thr Ala Ser Glu Leu Asp Pro Leu Val
260 265 270

Glu Gly Trp Leu Glu Glu His Lys Arg Lys Arg Ala Phe Asn Met Asp
275 280 285

Ala Lys Glu Glu Gln Asp Asn Phe Met Asp Val Met Leu Asn Val Leu
290 295 300

Lys Asp Ala Glu Ile Ser Gly Tyr Asp Ser Asp Thr Ile Ile Lys Ala
305 310 315 320

Thr Cys Leu Asn Leu Ile Leu Ala Gly Ser Asp Thr Thr Met Ile Ser
325 330 335

Leu Thr Trp Val Leu Ser Leu Leu Leu Asn His Gln Met Glu Leu Lys
340 345 350

Lys Val Gln Asp Glu Leu Asp Thr Tyr Ile Gly Lys Asp Arg Lys Val
355 360 365

Glu Glu Ser Asp Ile Thr Lys Leu Val Tyr Leu Gln Ala Ile Val Lys
370 375 380

Glu Thr Met Arg Leu Tyr Pro Pro Ser Pro Leu Ile Thr Leu Arg Ala
385 390 395 400

Ala Met Glu Asp Cys Thr Phe Ser Gly Gly Tyr His Ile Pro Ala Gly
405 410 415

Thr Arg Leu Met Val Asn Ala Trp Lys Ile His Arg Asp Gly Arg Val
420 425 430

Trp Ser Asp Pro His Asp Phe Lys Pro Gly Arg Phe Leu Thr Ser His
435 440 445

Lys Asp Val Asp Val Lys Gly Gln Asn Tyr Glu Leu Val Pro Phe Gly
450 455 460

Ser Gly Arg Arg Ala Cys Pro Gly Ala Ser Leu Ala Leu Arg Val Val
465 470 475 480

His Leu Thr Met Ala Arg Leu Leu His Ser Phe Asn Val Ala Ser Pro
485 490 495

Ser Asn Gln Val Val Asp Met Thr Glu Ser Ile Gly Leu Thr Asn Leu
500 505 510

```
Lys Ala Thr Pro Leu Glu Ile Leu Leu Thr Pro Arg Leu Asp Thr Lys
        515                 520                 525

Leu Tyr Glu Asn
    530
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1657 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1548

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
CTT GTT CTT CTT TCT CTA TTG TCT ATA GTC ATC TCC ATT GTT CTC TTC        48
Leu Val Leu Leu Ser Leu Leu Ser Ile Val Ile Ser Ile Val Leu Phe
  1               5                  10                  15

ATT ACC CAC ACA CAC AAA AGA AAC AAC ACT CCA AGA GGA CCA CCA GGT        96
Ile Thr His Thr His Lys Arg Asn Asn Thr Pro Arg Gly Pro Pro Gly
            20                  25                  30

CCT CCA CCT CTT CCT CTC ATC GGC AAC CTT CAC CAA CTC CAC AAC TCA       144
Pro Pro Pro Leu Pro Leu Ile Gly Asn Leu His Gln Leu His Asn Ser
        35                  40                  45

TCC CCA CAT CTC TGC CTA TGG CAA CTC GCC AAA CTC CAC GGT CCT CTC       192
Ser Pro His Leu Cys Leu Trp Gln Leu Ala Lys Leu His Gly Pro Leu
    50                  55                  60

ATG TCG TTT CGC CTC GGC GCC GTG CAA ACC GTC GTG GTT TCA TCG GCC       240
Met Ser Phe Arg Leu Gly Ala Val Gln Thr Val Val Val Ser Ser Ala
65                  70                  75                  80

AGA ATC GCC GAA CAA ATC TTG AAA ACC CAC GAC CTC AAC TTC GCT TCC       288
Arg Ile Ala Glu Gln Ile Leu Lys Thr His Asp Leu Asn Phe Ala Ser
                85                  90                  95

AGG CCT CTC TTC GTG GGC CCG AGA AAG CTC TCT TAC GAC GGG TTG GAC       336
Arg Pro Leu Phe Val Gly Pro Arg Lys Leu Ser Tyr Asp Gly Leu Asp
            100                 105                 110

ATG GGC TTC GCA CCG TAC GGC CCG TAC TGG AGA GAA ATG AAG AAA CTC       384
Met Gly Phe Ala Pro Tyr Gly Pro Tyr Trp Arg Glu Met Lys Lys Leu
        115                 120                 125

TGC ATC GTT CAC CTC TTC AGC GCG CAA CGC GTT CGG TCC TTT CGA CCA       432
Cys Ile Val His Leu Phe Ser Ala Gln Arg Val Arg Ser Phe Arg Pro
```

```
    130                 135                 140

ATT CGA GAG AAC GAG GTT GCA AAA ATG GTT CGG AAA CTG TCG GAA CAC      480
Ile Arg Glu Asn Glu Val Ala Lys Met Val Arg Lys Leu Ser Glu His
145                 150                 155                 160

GAA GCT TCG GGT ACT GTC GTG AAC TTG ACC GAA ACT TTG ATG TCT TTC      528
Glu Ala Ser Gly Thr Val Val Asn Leu Thr Glu Thr Leu Met Ser Phe
                165                 170                 175

ACG AAC TCT TTG ATA TGC ACA ATC GCC TTG GGG AAA AGT TAC GGT TGT      576
Thr Asn Ser Leu Ile Cys Arg Ile Ala Leu Gly Lys Ser Tyr Gly Cys
            180                 185                 190

GAG TAC GAG GAA GTA GTT GTT GAT GAG GTA CTG GGA AAC CGG AGG AGC      624
Glu Tyr Glu Glu Val Val Val Asp Glu Val Leu Gly Asn Arg Arg Ser
        195                 200                 205

AGG TTG CAG GTT CTG CTC AAC GAG GCT CAA GCG TTG CTT TCG GAG TTT      672
Arg Leu Gln Val Leu Leu Asn Glu Ala Gln Ala Leu Leu Ser Glu Phe
    210                 215                 220

TTC TTT TCG GAT TAT TTT CCG CCT ATA GGA AAG TGG GTT GAT AGA GTG      720
Phe Phe Ser Asp Tyr Phe Pro Pro Ile Gly Lys Trp Val Asp Arg Val
225                 230                 235                 240

ACG GGA ATT CTA TCG CGG CTT GAT AAA ACG TTC AAG GAG TTG GAC GCG      768
Thr Gly Ile Leu Ser Arg Leu Asp Lys Thr Phe Lys Glu Leu Asp Ala
                245                 250                 255

TGC TAC GAA CGA TCA TCC TAT GAT CAC ATG GAT TCG GCA AAG AGT GGT      816
Cys Tyr Glu Arg Ser Ser Tyr Asp His Met Asp Ser Ala Lys Ser Gly
            260                 265                 270

AAA AAA GAT AAT GAC AAC AAA GAA GTC AAA GAT ATT ATT GAT ATT CTT      864
Lys Lys Asp Asn Asp Asn Lys Glu Val Lys Asp Ile Ile Asp Ile Leu
        275                 280                 285

CTC CAG CTA CTT GAT GAT CGT TCC TTC ACC TTT GAT CTC ACT CTC GAC      912
Leu Gln Leu Leu Asp Asp Arg Ser Phe Thr Phe Asp Leu Thr Leu Asp
    290                 295                 300

CAC ATA AAA GCC GTG CTC ATG AAC ATC TTT ATA GCA GGA ACA GAC CCG      960
His Ile Lys Ala Val Leu Met Asn Ile Phe Ile Ala Gly Thr Asp Pro
305                 310                 315                 320

AGT TCC GCG ACA ATA GTT TGG GCA ATG AAT GCA CTG TTG AAG AAT CCC     1008
Ser Ser Ala Thr Ile Val Trp Ala Met Asn Ala Leu Leu Lys Asn Pro
                325                 330                 335

AAT GTG ATG AGC AAG GTT CAA GGA GAA GTG AGA AAT CTA TTC GGT GAC     1056
Asn Val Met Ser Lys Val Gln Gly Glu Val Arg Asn Leu Phe Gly Asp
            340                 345                 350

AAA GAT TTC ATA AAC GAA GAT GAT GTC GAA AGC CTT CCT TAT CTC AAA     1104
Lys Asp Phe Ile Asn Glu Asp Asp Val Glu Ser Leu Pro Tyr Leu Lys
        355                 360                 365

GCA GTG GTG AAG GAG ACA TTA AGA TTA TTC CCA CCT TCA CCA CTA CTT     1152
```

```
Ala Val Val Lys Glu Thr Leu Arg Leu Phe Pro Pro Ser Pro Leu Leu
    370                 375                 380

TTG CCA AGG GTA ACA ATG GAA ACA TGC AAC ATA GAA GGG TAC GAA ATT      1200
Leu Pro Arg Val Thr Met Glu Thr Cys Asn Ile Glu Gly Tyr Glu Ile
385                 390                 395                 400

CAA GCC AAA ACT ATA GTG CAT GTT AAT GCA TGG GCC ATA GCA AGG GAC      1248
Gln Ala Lys Thr Ile Val His Val Asn Ala Trp Ala Ile Ala Arg Asp
                405                 410                 415

CCT GAG AAT TGG GAA GAG CCT GAG AAA TTT TTC CCC GAA AGG TTC CTT      1296
Pro Glu Asn Trp Glu Glu Pro Glu Lys Phe Phe Pro Glu Arg Phe Leu
            420                 425                 430

GAG AGT TCG ATG GAG TTA AAG GGG AAT GAT GAG TTT AAG GTG ATC CCG      1344
Glu Ser Ser Met Glu Leu Lys Gly Asn Asp Glu Phe Lys Val Ile Pro
        435                 440                 445

TTT GGT TCT GGA AGG AGA ATG TGT CCT GCG AAG CAC ATG GGA ATT ATG      1392
Phe Gly Ser Gly Arg Arg Met Cys Pro Ala Lys His Met Gly Ile Met
    450                 455                 460

AAT GTT GAG CTT TCT CTT GCT AAT CTC ATT CAC ACG TTT GAT TGG GAA      1440
Asn Val Glu Leu Ser Leu Ala Asn Leu Ile His Thr Phe Asp Trp Glu
465                 470                 475                 480

GTG GCT AAA GGG TTC GAC AAG GAA GAA ATG TTG GAC ACG CAA ATG AAA      1488
Val Ala Lys Gly Phe Asp Lys Glu Glu Met Leu Asp Thr Gln Met Lys
                485                 490                 495

CCA GGA ATA ACG ATG CAC AAG AAA AGT GAT CTT TAC CTA GTG GCA AAG      1536
Pro Gly Ile Thr Met His Lys Lys Ser Asp Leu Tyr Leu Val Ala Lys
            500                 505                 510

AAA CCG ACA ACG TAGCACACGT TGGTACATTC ACTATAACAC ACAAGAAAGT          1588
Lys Pro Thr Thr
        515

TGATAATGAC TTGTGTATGC AACTATGCTC TATGCACTAT GCACTATGTT TATTGACCAT    1648

TAATTACTG                                                           1657
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 516 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Leu Val Leu Leu Ser Leu Leu Ser Ile Val Ile Ser Ile Val Leu Phe
  1               5                  10                  15

Ile Thr His Thr His Lys Arg Asn Asn Thr Pro Arg Gly Pro Pro Gly
            20                  25                  30
```

Pro Pro Pro Leu Pro Leu Ile Gly Asn Leu His Gln Leu His Asn Ser
35 40 45

Ser Pro His Leu Cys Leu Trp Gln Leu Ala Lys Leu His Gly Pro Leu
50 55 60

Met Ser Phe Arg Leu Gly Ala Val Gln Thr Val Val Val Ser Ser Ala
65 70 75 80

Arg Ile Ala Glu Gln Ile Leu Lys Thr His Asp Leu Asn Phe Ala Ser
85 90 95

Arg Pro Leu Phe Val Gly Pro Arg Lys Leu Ser Tyr Asp Gly Leu Asp
100 105 110

Met Gly Phe Ala Pro Tyr Gly Pro Tyr Trp Arg Glu Met Lys Lys Leu
115 120 125

Cys Ile Val His Leu Phe Ser Ala Gln Arg Val Arg Ser Phe Arg Pro
130 135 140

Ile Arg Glu Asn Glu Val Ala Lys Met Val Arg Lys Leu Ser Glu His
145 150 155 160

Glu Ala Ser Gly Thr Val Val Asn Leu Thr Glu Thr Leu Met Ser Phe
165 170 175

Thr Asn Ser Leu Ile Cys Arg Ile Ala Leu Gly Lys Ser Tyr Gly Cys
180 185 190

Glu Tyr Glu Glu Val Val Val Asp Glu Val Leu Gly Asn Arg Arg Ser
195 200 205

Arg Leu Gln Val Leu Leu Asn Glu Ala Gln Ala Leu Leu Ser Glu Phe
210 215 220

Phe Phe Ser Asp Tyr Phe Pro Pro Ile Gly Lys Trp Val Asp Arg Val
225 230 235 240

Thr Gly Ile Leu Ser Arg Leu Asp Lys Thr Phe Lys Glu Leu Asp Ala
245 250 255

Cys Tyr Glu Arg Ser Ser Tyr Asp His Met Asp Ser Ala Lys Ser Gly
260 265 270

Lys Lys Asp Asn Asp Asn Lys Glu Val Lys Asp Ile Ile Asp Ile Leu
275 280 285

Leu Gln Leu Leu Asp Asp Arg Ser Phe Thr Phe Asp Leu Thr Leu Asp
290 295 300

His Ile Lys Ala Val Leu Met Asn Ile Phe Ile Ala Gly Thr Asp Pro
305 310 315 320

Ser Ser Ala Thr Ile Val Trp Ala Met Asn Ala Leu Leu Lys Asn Pro
325 330 335

Asn Val Met Ser Lys Val Gln Gly Glu Val Arg Asn Leu Phe Gly Asp
340 345 350

```
Lys Asp Phe Ile Asn Glu Asp Asp Val Glu Ser Leu Pro Tyr Leu Lys
        355                 360                 365

Ala Val Val Lys Glu Thr Leu Arg Leu Phe Pro Pro Ser Pro Leu Leu
    370                 375                 380

Leu Pro Arg Val Thr Met Glu Thr Cys Asn Ile Glu Gly Tyr Glu Ile
385                 390                 395                 400

Gln Ala Lys Thr Ile Val His Val Asn Ala Trp Ala Ile Ala Arg Asp
                405                 410                 415

Pro Glu Asn Trp Glu Glu Pro Glu Lys Phe Phe Pro Glu Arg Phe Leu
            420                 425                 430

Glu Ser Ser Met Glu Leu Lys Gly Asn Asp Glu Phe Lys Val Ile Pro
        435                 440                 445

Phe Gly Ser Gly Arg Arg Met Cys Pro Ala Lys His Met Gly Ile Met
    450                 455                 460

Asn Val Glu Leu Ser Leu Ala Asn Leu Ile His Thr Phe Asp Trp Glu
465                 470                 475                 480

Val Ala Lys Gly Phe Asp Lys Glu Glu Met Leu Asp Thr Gln Met Lys
                485                 490                 495

Pro Gly Ile Thr Met His Lys Lys Ser Asp Leu Tyr Leu Val Ala Lys
            500                 505                 510

Lys Pro Thr Thr
        515
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1824 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 54..1616

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GGAAAATTAG CCTCACAAAA GCAAAGATCA AACAAACCAA GGACGAGAAC ACG ATG          56
                                                           Met
                                                            1

TTG CTT GAA CTT GCA CTT GGT TTA TTG GTT TTG GCT CTG TTT CTG CAC          104
Leu Leu Glu Leu Ala Leu Gly Leu Leu Val Leu Ala Leu Phe Leu His
  5                  10                  15
```

```
TTG CGT CCC ACA CCC ACT GCA AAA TCA AAA GCA CTT CGC CAT CTC CCA       152
Leu Arg Pro Thr Pro Thr Ala Lys Ser Lys Ala Leu Arg His Leu Pro
         20                  25                  30

AAC CCA CCA AGC CCA AAG CCT CGT CTT CCC TTC ATA GGA CAC CTT CAT       200
Asn Pro Pro Ser Pro Lys Pro Arg Leu Pro Phe Ile Gly His Leu His
     35                  40                  45

CTC TTA AAA GAC AAA CTT CTC CAC TAC GCA CTC ATC GAC CTC TCC AAA       248
Leu Leu Lys Asp Lys Leu Leu His Tyr Ala Leu Ile Asp Leu Ser Lys
 50                  55                  60                  65

AAA CAT GGT CCC TTA TTC TCT CTC TAC TTT GGC TCC ATG CCA ACC GTT       296
Lys His Gly Pro Leu Phe Ser Leu Tyr Phe Gly Ser Met Pro Thr Val
                 70                  75                  80

GTT GCC TCC ACA CCA GAA TTG TTC AAG CTC TTC CTC CAA ACG CAC GAG       344
Val Ala Ser Thr Pro Glu Leu Phe Lys Leu Phe Leu Gln Thr His Glu
             85                  90                  95

GCA ACT TCC TTC AAC ACA AGG TTC CAA ACC TCA GCC ATA AGA CGC CTC       392
Ala Thr Ser Phe Asn Thr Arg Phe Gln Thr Ser Ala Ile Arg Arg Leu
         100                 105                 110

ACC TAT GAT AGC TCA GTG GCC ATG GTT CCC TTC GGA CCT TAC TGG AAG       440
Thr Tyr Asp Ser Ser Val Ala Met Val Pro Phe Gly Pro Tyr Trp Lys
     115                 120                 125

TTC GTG AGG AAG CTC ATC ATG AAC GAC CTT CCC AAC GCC ACC ACT GTA       488
Phe Val Arg Lys Leu Ile Met Asn Asp Leu Pro Asn Ala Thr Thr Val
130                 135                 140                 145

AAC AAG TTG AGG CCT TTG AGG ACC CAA CAG ACC CGC AAG TTC CTT AGG       536
Asn Lys Leu Arg Pro Leu Arg Thr Gln Gln Thr Arg Lys Phe Leu Arg
                150                 155                 160

GTT ATG GCC CAA GGC GCA GAG GCA CAG AAG CCC CTT GAC TTG ACC GAG       584
Val Met Ala Gln Gly Ala Glu Ala Gln Lys Pro Leu Asp Leu Thr Glu
            165                 170                 175

GAG CTT CTG AAA TGG ACC AAC AGC ACC ATC TCC ATG ATG ATG CTC GGC       632
Glu Leu Leu Lys Trp Thr Asn Ser Thr Ile Ser Met Met Met Leu Gly
        180                 185                 190

GAG GCT GAG GAG ATC AGA GAC ATC GCT CGC GAG GTT CTT AAG ATC TTT       680
Glu Ala Glu Glu Ile Arg Asp Ile Ala Arg Glu Val Leu Lys Ile Phe
    195                 200                 205

GGC GAA TAC AGC CTC ACT GAC TTC ATC TGG CCA TTG AAG CAT CTC AAG       728
Gly Glu Tyr Ser Leu Thr Asp Phe Ile Trp Pro Leu Lys His Leu Lys
210                 215                 220                 225

GTT GGA AAG TAT GAG AAG AGG ATC GAC GAC ATC TTG AAC AAG TTC GAC       776
Val Gly Lys Tyr Glu Lys Arg Ile Asp Asp Ile Leu Asn Lys Phe Asp
                230                 235                 240

CCT GTC GTT GAA AGG GTC ATC AAG AAG CGC CGT GAG ATC GTG AGG AGG       824
Pro Val Val Glu Arg Val Ile Lys Lys Arg Arg Glu Ile Val Arg Arg
            245                 250                 255
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AGA AAG AAC GGA GAG GTT GTT GAG GGT GAG GTC AGC GGG GTT TTC CTT        872
Arg Lys Asn Gly Glu Val Val Glu Gly Glu Val Ser Gly Val Phe Leu
        260                 265                 270

GAC ACT TTG CTT GAA TTC GCT GAG GAT GAG ACC ATG GAG ATC AAA ATC        920
Asp Thr Leu Leu Glu Phe Ala Glu Asp Glu Thr Met Glu Ile Lys Ile
    275                 280                 285

ACC AAG GAC CAC ATC GAG GGT CTT GTT GTC GAC TTT TTC TCG GCA GGA        968
Thr Lys Asp His Ile Glu Gly Leu Val Val Asp Phe Phe Ser Ala Gly
290                 295                 300                 305

ACA GAC TCC ACA GCG GTG GCA ACA GAG TGG GCA TTG GCA GAA CTC ATC       1016
Thr Asp Ser Thr Ala Val Ala Thr Glu Trp Ala Leu Ala Glu Leu Ile
                310                 315                 320

AAC AAT CCT AAG GTG TTG GAA AAG GCT CGT GAG GAG GTC TAC AGT GTT       1064
Asn Asn Pro Lys Val Leu Glu Lys Ala Arg Glu Glu Val Tyr Ser Val
            325                 330                 335

GTG GGA AAG GAC AGA CTT GTG GAC GAA GTT GAC ACT CAA AAC CTT CCT       1112
Val Gly Lys Asp Arg Leu Val Asp Glu Val Asp Thr Gln Asn Leu Pro
        340                 345                 350

TAC ATT AGA GCA ATC GTG AAG GAG ACA TTC CGC ATG CAC CCG CCA CTC       1160
Tyr Ile Arg Ala Ile Val Lys Glu Thr Phe Arg Met His Pro Pro Leu
    355                 360                 365

CCA GTG GTC AAA AGA AAG TGC ACA GAA GAG TGT GAG ATT AAT GGA TAT       1208
Pro Val Val Lys Arg Lys Cys Thr Glu Glu Cys Glu Ile Asn Gly Tyr
370                 375                 380                 385

GTG ATC CCA GAG GGA GCA TTG ATT CTC TTC AAT GTA TGG CAA GTA GGA       1256
Val Ile Pro Glu Gly Ala Leu Ile Leu Phe Asn Val Trp Gln Val Gly
                390                 395                 400

AGA GAC CCC AAA TAC TGG GAC AGA CCA TCG GAG TTC CGT CCT GAG AGG       1304
Arg Asp Pro Lys Tyr Trp Asp Arg Pro Ser Glu Phe Arg Pro Glu Arg
            405                 410                 415

TTC CTA GAG ACA GGG GCT GAA GGG GAA GCA GGG CCT CTT GAT CTT AGG       1352
Phe Leu Glu Thr Gly Ala Glu Gly Glu Ala Gly Pro Leu Asp Leu Arg
        420                 425                 430

GGA CAA CAT TTT CAA CTT CTC CCA TTT GGG TCT GGG AGG AGA ATG TGC       1400
Gly Gln His Phe Gln Leu Leu Pro Phe Gly Ser Gly Arg Arg Met Cys
    435                 440                 445

CCT GGA GTC AAT CTG GCT ACT TCG GGA ATG GCA ACA CTT CTT GCA TCT       1448
Pro Gly Val Asn Leu Ala Thr Ser Gly Met Ala Thr Leu Leu Ala Ser
450                 455                 460                 465

CTT ATT CAG TGC TTC GAC TTG CAA GTG CTG GGT CCA CAA GGA CAG ATA       1496
Leu Ile Gln Cys Phe Asp Leu Gln Val Leu Gly Pro Gln Gly Gln Ile
                470                 475                 480

TTG AAG GGT GGT GAC GCC AAA GTT AGC ATG GAA GAG AGA GCC GGC CTC       1544
Leu Lys Gly Gly Asp Ala Lys Val Ser Met Glu Glu Arg Ala Gly Leu
            485                 490                 495
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ACT GTT CCA AGG GCA CAT AGT CTT GTC TGT GTT CCA CTT GCA AGG ATC      1592
Thr Val Pro Arg Ala His Ser Leu Val Cys Val Pro Leu Ala Arg Ile
        500                 505                 510

GGC GTT GCA TCT AAA CTC CTT TCT TAATTAAGAT CATCATCATA TATAATATTT     1646
Gly Val Ala Ser Lys Leu Leu Ser
    515                 520

ACTTTTTGTG TGTTGATAAT CATCATTTCA ATAAGGTCTC GTTCATCTAC TTTTTATGAA    1706

GTATATAAGC CCTTCCATGC ACATTGTATC ATCTCCCATT TGTCTTCGTT TGCTACCTAA    1766

GGCAATCTTT TTTTTTTTAG AATCACATCA TCCTACTATA AACTATCAAT CCTTATAT      1824
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(2) INFORMATION FOR SEQ ID NO.14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 521 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Leu Leu Glu Leu Ala Leu Gly Leu Leu Val Leu Ala Leu Phe Leu
 1               5                  10                  15

His Leu Arg Pro Thr Pro Thr Ala Lys Ser Lys Ala Leu Arg His Leu
            20                  25                  30

Pro Asn Pro Pro Ser Pro Lys Pro Arg Leu Pro Phe Ile Gly His Leu
        35                  40                  45

His Leu Leu Lys Asp Lys Leu Leu His Tyr Ala Leu Ile Asp Leu Ser
    50                  55                  60

Lys Lys His Gly Pro Leu Phe Ser Leu Tyr Phe Gly Ser Met Pro Thr
65                  70                  75                  80

Val Val Ala Ser Thr Pro Glu Leu Phe Lys Leu Phe Leu Gln Thr His
                85                  90                  95

Glu Ala Thr Ser Phe Asn Thr Arg Phe Gln Thr Ser Ala Ile Arg Arg
            100                 105                 110

Leu Thr Tyr Asp Ser Ser Val Ala Met Val Pro Phe Gly Pro Tyr Trp
        115                 120                 125

Lys Phe Val Arg Lys Leu Ile Met Asn Asp Leu Pro Asn Ala Thr Thr
    130                 135                 140

Val Asn Lys Leu Arg Pro Leu Arg Thr Gln Gln Thr Arg Lys Phe Leu
145                 150                 155                 160

Arg Val Met Ala Gln Gly Ala Glu Ala Gln Lys Pro Leu Asp Leu Thr
                165                 170                 175

Glu Glu Leu Leu Lys Trp Thr Asn Ser Thr Ile Ser Met Met Met Leu
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180 185 190

Gly Glu Ala Glu Glu Ile Arg Asp Ile Ala Arg Glu Val Leu Lys Ile
195 200 205

Phe Gly Glu Tyr Ser Leu Thr Asp Phe Ile Trp Pro Leu Lys His Leu
210 215 220

Lys Val Gly Lys Tyr Glu Lys Arg Ile Asp Asp Ile Leu Asn Lys Phe
225 230 235 240

Asp Pro Val Val Glu Arg Val Ile Lys Lys Arg Arg Glu Ile Val Arg
245 250 255

Arg Arg Lys Asn Gly Glu Val Val Glu Gly Glu Val Ser Gly Val Phe
260 265 270

Leu Asp Thr Leu Leu Glu Phe Ala Glu Asp Glu Thr Met Glu Ile Lys
275 280 285

Ile Thr Lys Asp His Ile Glu Gly Leu Val Val Asp Phe Phe Ser Ala
290 295 300

Gly Thr Asp Ser Thr Ala Val Ala Thr Glu Trp Ala Leu Ala Glu Leu
305 310 315 320

Ile Asn Asn Pro Lys Val Leu Glu Lys Ala Arg Glu Glu Val Tyr Ser
325 330 335

Val Val Gly Lys Asp Arg Leu Val Asp Glu Val Asp Thr Gln Asn Leu
340 345 350

Pro Tyr Ile Arg Ala Ile Val Lys Glu Thr Phe Arg Met His Pro Pro
355 360 365

Leu Pro Val Val Lys Arg Lys Cys Thr Glu Glu Cys Glu Ile Asn Gly
370 375 380

Tyr Val Ile Pro Glu Gly Ala Leu Ile Leu Phe Asn Val Trp Gln Val
385 390 395 400

Gly Arg Asp Pro Lys Tyr Trp Asp Arg Pro Ser Glu Phe Arg Pro Glu
405 410 415

Arg Phe Leu Glu Thr Gly Ala Glu Gly Glu Ala Gly Pro Leu Asp Leu
420 425 430

Arg Gly Gln His Phe Gln Leu Leu Pro Phe Gly Ser Gly Arg Arg Met
435 440 445

Cys Pro Gly Val Asn Leu Ala Thr Ser Gly Met Ala Thr Leu Leu Ala
450 455 460

Ser Leu Ile Gln Cys Phe Asp Leu Gln Val Leu Gly Pro Gln Gly Gln
465 470 475 480

Ile Leu Lys Gly Gly Asp Ala Lys Val Ser Met Glu Glu Arg Ala Gly
485 490 495

Leu Thr Val Pro Arg Ala His Ser Leu Val Cys Val Pro Leu Ala Arg

500 505 510

Ile Gly Val Ala Ser Lys Leu Leu Ser
515 520

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1831 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 20..1747

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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CAACACTCGC AGTACCGCC ATG AGT GTC GAC ACT TCC TCC ACC CTC TCC ACC        52
                      Met Ser Val Asp Thr Ser Ser Thr Leu Ser Thr
                        1               5                  10

GTC ACC GAT GCC AAT CTT CAC TCC AGA TTT CAT TCT CGT CTT GTT CCA       100
Val Thr Asp Ala Asn Leu His Ser Arg Phe His Ser Arg Leu Val Pro
            15                  20                  25

TTC ACT CAT CAT TTC TCA CTT TCT CAA CCC AAA CGG ATT TCT TCA ATC       148
Phe Thr His His Phe Ser Leu Ser Gln Pro Lys Arg Ile Ser Ser Ile
        30                  35                  40

AGA TGC CAA TCA ATT AAT ACC GAT AAG AAG AAA TCA AGT AGA AAT CTG       196
Arg Cys Gln Ser Ile Asn Thr Asp Lys Lys Lys Ser Ser Arg Asn Leu
    45                  50                  55

CTG GGC AAT GCA AGT AAC CTC CTC ACG GAC TTA TTA AGT GGT GGA AGT       244
Leu Gly Asn Ala Ser Asn Leu Leu Thr Asp Leu Leu Ser Gly Gly Ser
60                  65                  70                  75

ATA GGG TCT ATG CCC ATA GCT GAA GGT GCA GTC TCA GAT CTG CTT GGT       292
Ile Gly Ser Met Pro Ile Ala Glu Gly Ala Val Ser Asp Leu Leu Gly
                80                  85                  90

CGA CCT CTC TTT TTC TCA CTG TAT GAT TGG TTC TTG GAG CAT GGT GCG       340
Arg Pro Leu Phe Phe Ser Leu Tyr Asp Trp Phe Leu Glu His Gly Ala
            95                  100                 105

GTG TAT AAA CTT GCC TTT GGA CCA AAA GCA TTT GTT GTT GTA TCA GAT       388
Val Tyr Lys Leu Ala Phe Gly Pro Lys Ala Phe Val Val Val Ser Asp
        110                 115                 120

CCC ATA GTT GCT AGA CAT ATT CTG CGA GAA AAT GCA TTT TCT TAT GAC       436
Pro Ile Val Ala Arg His Ile Leu Arg Glu Asn Ala Phe Ser Tyr Asp
    125                 130                 135

AAG GGA GTA CTT GCT GAT ATC CTT GAA CCA ATA ATG GGC AAA GGA CTC       484
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Lys Gly Val Leu Ala Asp Ile Leu Glu Pro Ile Met Gly Lys Gly Leu
140                 145                 150                 155

ATA CCA GCA GAC CTT GAT ACT TGG AAG CAA AGG AGA AGA GTC ATT GCT      532
Ile Pro Ala Asp Leu Asp Thr Trp Lys Gln Arg Arg Arg Val Ile Ala
                160                 165                 170

CCG GCT TTC CAT AAC TCA TAC TTG GAA GCT ATG GTT AAA ATA TTC ACA      580
Pro Ala Phe His Asn Ser Tyr Leu Glu Ala Met Val Lys Ile Phe Thr
            175                 180                 185

ACT TGT TCA GAA AGA ACA ATA TTG AAG TTT AAT AAG CTT CTT GAA GGA      628
Thr Cys Ser Glu Arg Thr Ile Leu Lys Phe Asn Lys Leu Leu Glu Gly
        190                 195                 200


GAG GGT TAT GAT GGA CCT GAC TCA ATT GAA TTG GAT CTT GAG GCA GAG      676
Glu Gly Tyr Asp Gly Pro Asp Ser Ile Glu Leu Asp Leu Glu Ala Glu
    205                 210                 215

TTT TCT AGT TTG GCT CTT GAT ATT ATT GGG CTT GGT GTG TTC AAC TAT      724
Phe Ser Ser Leu Ala Leu Asp Ile Ile Gly Leu Gly Val Phe Asn Tyr
220                 225                 230                 235

GAC TTT GGT TCT GTC ACC AAA GAA TCT CCA GTT ATT AAG GCA GTC TAT      772
Asp Phe Gly Ser Val Thr Lys Glu Ser Pro Val Ile Lys Ala Val Tyr
                240                 245                 250

GGC ACT CTT TTT GAA GCT GAA CAC AGA TCC ACT TTC TAC ATT CCA TAT      820
Gly Thr Leu Phe Glu Ala Glu His Arg Ser Thr Phe Tyr Ile Pro Tyr
            255                 260                 265

TGG AAA ATT CCA TTG GCA AGG TGG ATA GTC CCA AGG CAA AGA AAG TTT      868
Trp Lys Ile Pro Leu Ala Arg Trp Ile Val Pro Arg Gln Arg Lys Phe
        270                 275                 280

CAG GAT GAC CTA AAG GTC ATC AAT ACT TGT CTT GAT GGA CTT ATC AGA      916
Gln Asp Asp Leu Lys Val Ile Asn Thr Cys Leu Asp Gly Leu Ile Arg
    285                 290                 295

AAT GCA AAA GAG AGC AGA CAG GAA ACA GAT GTT GAG AAA TTG CAG CAG      964
Asn Ala Lys Glu Ser Arg Gln Glu Thr Asp Val Glu Lys Leu Gln Gln
300                 305                 310                 315

AGG GAT TAC TTA AAT TTG AAG GAT GCA AGT CTT CTG CGT TTC CTG GTT     1012
Arg Asp Tyr Leu Asn Leu Lys Asp Ala Ser Leu Leu Arg Phe Leu Val
                320                 325                 330

GAT ATG CGG GGA GCT GAT GTT GAT GAT CGT CAG TTG AGG GAT GAT TTA     1060
Asp Met Arg Gly Ala Asp Val Asp Asp Arg Gln Leu Arg Asp Asp Leu
            335                 340                 345

ATG ACA ATG CTT ATT GCC GGT CAT GAA ACA ACG GCT GCA GTT CTT ACT     1108
Met Thr Met Leu Ile Ala Gly His Glu Thr Thr Ala Ala Val Leu Thr
        350                 355                 360

TGG GCA GTT TTC CTC CTA GCT CAA AAT CCT AGC AAA ATG AAG AAG GCT     1156
Trp Ala Val Phe Leu Leu Ala Gln Asn Pro Ser Lys Met Lys Lys Ala
    365                 370                 375
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CAA GCA GAG GTA GAT TTG GTG CTG GGT ACG GGG AGG CCA ACT TTT GAA      1204
Gln Ala Glu Val Asp Leu Val Leu Gly Thr Gly Arg Pro Thr Phe Glu
380                 385                 390                 395

TCA CTT AAG GAA TTG CAG TAC ATT AGA TTG ATT GTT GTG GAG GCT CTT      1252
Ser Leu Lys Glu Leu Gln Tyr Ile Arg Leu Ile Val Val Glu Ala Leu
                400                 405                 410

CGT TTA TAC CCC CAA CCA CCT TTG CTG ATT AGA CGT TCA CTC AAA TCT      1300
Arg Leu Tyr Pro Gln Pro Pro Leu Leu Ile Arg Arg Ser Leu Lys Ser
            415                 420                 425

GAT GTT TTA CCA GGT GGG CAC AAA GGT GAA AAA GAT GGT TAT GCA ATT      1348
Asp Val Leu Pro Gly Gly His Lys Gly Glu Lys Asp Gly Tyr Ala Ile
        430                 435                 440

CCT GCT GGG ACT GAT GTC TTC ATT TCT GTA TAT AAT CTC CAT AGA TCT      1396
Pro Ala Gly Thr Asp Val Phe Ile Ser Val Tyr Asn Leu His Arg Ser
    445                 450                 455

CCA TAT TTT TGG GAC CGC CCT GAT GAC TTC GAA CCA GAG AGA TTT CTT      1444
Pro Tyr Phe Trp Asp Arg Pro Asp Asp Phe Glu Pro Glu Arg Phe Leu
460                 465                 470                 475

GTG CAA AAC AAG AAT GAA GAA ATT GAA GGA TGG GCT GGT CTT GAT CCA      1492
Val Gln Asn Lys Asn Glu Glu Ile Glu Gly Trp Ala Gly Leu Asp Pro
                480                 485                 490

TCT CGA AGT CCC GGA GCC TTG TAT CCG AAC GAG GTT ATA TCG GAT TTT      1540
Ser Arg Ser Pro Gly Ala Leu Tyr Pro Asn Glu Val Ile Ser Asp Phe
            495                 500                 505

GCA TTC TTA CCT TTT GGT GGC GGA CCA CGA AAA TGT GTT GGG GAC CAA      1588
Ala Phe Leu Pro Phe Gly Gly Gly Pro Arg Lys Cys Val Gly Asp Gln
        510                 515                 520

TTT GCT CTG ATG GAG TCC ACT GTA GCG TTG ACT ATG CTG CTC CAG AAT      1636
Phe Ala Leu Met Glu Ser Thr Val Ala Leu Thr Met Leu Leu Gln Asn
    525                 530                 535

TTT GAC GTG GAA CTA AAA GGG ACC CCT GAA TCG GTG GAA CTA GTT ACT      1684
Phe Asp Val Glu Leu Lys Gly Thr Pro Glu Ser Val Glu Leu Val Thr
540                 545                 550                 555

GGG GCA ACT ATT CAT ACC AAA AAT GGA ATG TGG TGC AGA TTG AAG AAG      1732
Gly Ala Thr Ile His Thr Lys Asn Gly Met Trp Cys Arg Leu Lys Lys
                560                 565                 570

AGA TCT AAT TTA CGT TGACATATGT ACTGTGGCCA TTTTTCTTAT ACAGAATAAT      1787
Arg Ser Asn Leu Arg
            575

GTATATTATT ATTCTTTGAG AATAATATGA ATAAATTCCT AGAC                     1831
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(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 576 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Met Ser Val Asp Thr Ser Ser Thr Leu Ser Thr Val Thr Asp Ala Asn
 1               5                  10                  15

Leu His Ser Arg Phe His Ser Arg Leu Val Pro Phe Thr His His Phe
            20                  25                  30

Ser Leu Ser Gln Pro Lys Arg Ile Ser Ser Ile Arg Cys Gln Ser Ile
        35                  40                  45

Asn Thr Asp Lys Lys Lys Ser Ser Arg Asn Leu Leu Gly Asn Ala Ser
    50                  55                  60

Asn Leu Leu Thr Asp Leu Leu Ser Gly Gly Ser Ile Gly Ser Met Pro
65                  70                  75                  80

Ile Ala Glu Gly Ala Val Ser Asp Leu Leu Gly Arg Pro Leu Phe Phe
                85                  90                  95

Ser Leu Tyr Asp Trp Phe Leu Glu His Gly Ala Val Tyr Lys Leu Ala
            100                 105                 110

Phe Gly Pro Lys Ala Phe Val Val Val Ser Asp Pro Ile Val Ala Arg
        115                 120                 125

His Ile Leu Arg Glu Asn Ala Phe Ser Tyr Asp Lys Gly Val Leu Ala
    130                 135                 140

Asp Ile Leu Glu Pro Ile Met Gly Lys Gly Leu Ile Pro Ala Asp Leu
145                 150                 155                 160

Asp Thr Trp Lys Gln Arg Arg Arg Val Ile Ala Pro Ala Phe His Asn
                165                 170                 175

Ser Tyr Leu Glu Ala Met Val Lys Ile Phe Thr Thr Cys Ser Glu Arg
            180                 185                 190

Thr Ile Leu Lys Phe Asn Lys Leu Leu Glu Gly Glu Gly Tyr Asp Gly
        195                 200                 205

Pro Asp Ser Ile Glu Leu Asp Leu Glu Ala Glu Phe Ser Ser Leu Ala
    210                 215                 220

Leu Asp Ile Ile Gly Leu Gly Val Phe Asn Tyr Asp Phe Gly Ser Val
225                 230                 235                 240

Thr Lys Glu Ser Pro Val Ile Lys Ala Val Tyr Gly Thr Leu Phe Glu
                245                 250                 255

Ala Glu His Arg Ser Thr Phe Tyr Ile Pro Tyr Trp Lys Ile Pro Leu
            260                 265                 270

Ala Arg Trp Ile Val Pro Arg Gln Arg Lys Phe Gln Asp Asp Leu Lys
        275                 280                 285
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Val Ile Asn Thr Cys Leu Asp Gly Leu Ile Arg Asn Ala Lys Glu Ser
290 295 300

Arg Gln Glu Thr Asp Val Glu Lys Leu Gln Gln Arg Asp Tyr Leu Asn
305 310 315 320

Leu Lys Asp Ala Ser Leu Leu Arg Phe Leu Val Asp Met Arg Gly Ala
325 330 335

Asp Val Asp Asp Arg Gln Leu Arg Asp Asp Leu Met Thr Met Leu Ile
340 345 350

Ala Gly His Glu Thr Thr Ala Ala Val Leu Thr Trp Ala Val Phe Leu
355 360 365

Leu Ala Gln Asn Pro Ser Lys Met Lys Lys Ala Gln Ala Glu Val Asp
370 375 380

Leu Val Leu Gly Thr Gly Arg Pro Thr Phe Glu Ser Leu Lys Glu Leu
385 390 395 400

Gln Tyr Ile Arg Leu Ile Val Val Glu Ala Leu Arg Leu Tyr Pro Gln
405 410 415

Pro Pro Leu Leu Ile Arg Arg Ser Leu Lys Ser Asp Val Leu Pro Gly
420 425 430

Gly His Lys Gly Glu Lys Asp Gly Tyr Ala Ile Pro Ala Gly Thr Asp
435 440 445

Val Phe Ile Ser Val Tyr Asn Leu His Arg Ser Pro Tyr Phe Trp Asp
450 455 460

Arg Pro Asp Asp Phe Glu Pro Glu Arg Phe Leu Val Gln Asn Lys Asn
465 470 475 480

Glu Glu Ile Glu Gly Trp Ala Gly Leu Asp Pro Ser Arg Ser Pro Gly
485 490 495

Ala Leu Tyr Pro Asn Glu Val Ile Ser Asp Phe Ala Phe Leu Pro Phe
500 505 510

Gly Gly Gly Pro Arg Lys Cys Val Gly Asp Gln Phe Ala Leu Met Glu
515 520 525

Ser Thr Val Ala Leu Thr Met Leu Leu Gln Asn Phe Asp Val Glu Leu
530 535 540

Lys Gly Thr Pro Glu Ser Val Glu Leu Val Thr Gly Ala Thr Ile His
545 550 555 560

Thr Lys Asn Gly Met Trp Cys Arg Leu Lys Lys Arg Ser Asn Leu Arg
565 570 575

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1704 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 38..1564

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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CAGGCTCCAC AAAACATCTC ATCATTCACC CAACAAA ATG GCG CTG CTT CTG ATA        55
                                          Met Ala Leu Leu Leu Ile
                                            1               5

ATT CCC ATC TCA CTG GTC ACC CTC TGG CTC GGT TAC ACC CTA TAC CAG       103
Ile Pro Ile Ser Leu Val Thr Leu Trp Leu Gly Tyr Thr Leu Tyr Gln
            10                  15                  20

CGA TTA AGA TTC AAG CTC CCT CCG GGT CCA CGG CCC TGG CCG GTA GTC       151
Arg Leu Arg Phe Lys Leu Pro Pro Gly Pro Arg Pro Trp Pro Val Val
        25                  30                  35

GGT AAC CTC TAC GAC ATA AAA CCC GTC CGC TTC CGG TGC TTC GCG GAG       199
Gly Asn Leu Tyr Asp Ile Lys Pro Val Arg Phe Arg Cys Phe Ala Glu
    40                  45                  50

TGG GCG CAG TCT TAC GGC CCC ATA ATA TCG GTT TGG TTC GGT TCG ACC       247
Trp Ala Gln Ser Tyr Gly Pro Ile Ile Ser Val Trp Phe Gly Ser Thr
55                  60                  65                  70

CTA AAC GTC ATC GTT TCG AAC TCG GAG CTG GCG AAG GAG GTG CTG AAG       295
Leu Asn Val Ile Val Ser Asn Ser Glu Leu Ala Lys Glu Val Leu Lys
                75                  80                  85

GAG CAC GAT CAG CTG CTG GCG GAC CGC CAC CGG AGC CGG TCG GCG GCG       343
Glu His Asp Gln Leu Leu Ala Asp Arg His Arg Ser Arg Ser Ala Ala
            90                  95                  100

AAG TTC AGC CGC GAC GGG AAG GAT CTA ATT TGG GCC GAT TAT GGG CCG       391
Lys Phe Ser Arg Asp Gly Lys Asp Leu Ile Trp Ala Asp Tyr Gly Pro
        105                 110                 115

CAC TAC GTG AAG GTG AGG AAG GTT TGC ACG CTC GAG CTT TTC TCG CCG       439
His Tyr Val Lys Val Arg Lys Val Cys Thr Leu Glu Leu Phe Ser Pro
    120                 125                 130

AAG CGC CTC GAG GCC CTG AGG CCC ATT AGG GAG GAC GAG GTC ACC TCC       487
Lys Arg Leu Glu Ala Leu Arg Pro Ile Arg Glu Asp Glu Val Thr Ser
135                 140                 145                 150

ATG GTT GAC TCC GTT TAC AAT CAC TGC ACC AGC ACT GAA AAT TTG GGG       535
Met Val Asp Ser Val Tyr Asn His Cys Thr Ser Thr Glu Asn Leu Gly
                155                 160                 165

AAA GGA ATA TTG TTG AGG AAG CAC TTG GGG GTT GTG GCA TTC AAC AAC       583
Lys Gly Ile Leu Leu Arg Lys His Leu Gly Val Val Ala Phe Asn Asn
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                170                 175                 180

ATA ACC AGG TTG GCA TTT GGG AAA AGA TTT GTG AAC TCA GAA GGT GTG      631
Ile Thr Arg Leu Ala Phe Gly Lys Arg Phe Val Asn Ser Glu Gly Val
        185                 190                 195

ATG GAT GAG CAA GGA GTA GAA TTC AAG GCC ATT GTG GAA AAT GGG TTA      679
Met Asp Glu Gln Gly Val Glu Phe Lys Ala Ile Val Glu Asn Gly Leu
    200                 205                 210

AAG CTA GGA GCA TCT CTA GCC ATG GCA GAA CAC ATC CCT TGG CTT CGC      727
Lys Leu Gly Ala Ser Leu Ala Met Ala Glu His Ile Pro Trp Leu Arg
215                 220                 225                 230

TGG ATG TTC CCA CTG GAA GAA GGA GCT TTT GCC AAG CAT GGA GCC CGC      775
Trp Met Phe Pro Leu Glu Glu Gly Ala Phe Ala Lys His Gly Ala Arg
                235                 240                 245

CGC GAC CGA CTC ACC AGA GCC ATC ATG GCA GAG CAC ACT GAA GCA CGC      823
Arg Asp Arg Leu Thr Arg Ala Ile Met Ala Glu His Thr Glu Ala Arg
            250                 255                 260

AAG AAA TCT GGT GGT GCC AAG CAA CAT TTT GTT GAT GCC CTC CTC ACA      871
Lys Lys Ser Gly Gly Ala Lys Gln His Phe Val Asp Ala Leu Leu Thr
        265                 270                 275

TTG CAA GAC AAA TAT GAC CTT AGT GAA GAC ACC ATC ATT GGT CTC CTT      919
Leu Gln Asp Lys Tyr Asp Leu Ser Glu Asp Thr Ile Ile Gly Leu Leu
    280                 285                 290

TGG GAT ATG ATC ACA GCA GGG ATG GAC ACA ACT GCA ATT TCA GTT GAG      967
Trp Asp Met Ile Thr Ala Gly Met Asp Thr Thr Ala Ile Ser Val Glu
295                 300                 305                 310

TGG GCC ATG GCT GAG TTG ATA AGA AAC CCA AGG GTG CAA CAA AAG GTC     1015
Trp Ala Met Ala Glu Leu Ile Arg Asn Pro Arg Val Gln Gln Lys Val
                315                 320                 325

CAA GAG GAG CTA GAC AGG GTA ATT GGG CTT GAA AGG GTG ATG ACT GAA     1063
Gln Glu Glu Leu Asp Arg Val Ile Gly Leu Glu Arg Val Met Thr Glu
            330                 335                 340

GCA GAC TTC TCA AAT CTC CCT TAC CTA CAA TGT GTG ACC AAA GAA GCA     1111
Ala Asp Phe Ser Asn Leu Pro Tyr Leu Gln Cys Val Thr Lys Glu Ala
        345                 350                 355

ATG AGG CTT CAC CCA CCA ACC CCA CTA ATG CTC CCA CAC CGT GCC AAT     1159
Met Arg Leu His Pro Pro Thr Pro Leu Met Leu Pro His Arg Ala Asn
    360                 365                 370

GCC AAT GTC AAA GTT GGA GGC TAT GAC ATT CCC AAA GGG TCC AAT GTG     1207
Ala Asn Val Lys Val Gly Gly Tyr Asp Ile Pro Lys Gly Ser Asn Val
375                 380                 385                 390

CAT GTG AAT GTG TGG GCG GTG GCC CGC GAC CCG GCC GTG TGG AAG GAT     1255
His Val Asn Val Trp Ala Val Ala Arg Asp Pro Ala Val Trp Lys Asp
                395                 400                 405

CCA TTG GAG TTC CGA CCC GAA AGG TTC CTT GAG GAG GAT GTA GAC ATG     1303
Pro Leu Glu Phe Arg Pro Glu Arg Phe Leu Glu Glu Asp Val Asp Met
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            410                 415                 420

AAG GGC CAT GAC TTT AGG CTA CTT CCA TTC GGG TCG GGT CGA CGA GTA      1351
Lys Gly His Asp Phe Arg Leu Leu Pro Phe Gly Ser Gly Arg Arg Val
        425                 430                 435

TGC CCG GGT GCC CAA CTT GGT ATC AAC TTG GCA GCA TCC ATG TTG GGC      1399
Cys Pro Gly Ala Gln Leu Gly Ile Asn Leu Ala Ala Ser Met Leu Gly
    440                 445                 450

CAC CTC TTG CAC CAT TTC TGT TGG ACC CCA CCT GAA GGA ATG AAG CCT      1447
His Leu Leu His His Phe Cys Trp Thr Pro Pro Glu Gly Met Lys Pro
455                 460                 465                 470

GAG GAA ATT GAC ATG GGA GAG AAT CCA GGG CTA GTC ACA TAC ATG AGG      1495
Glu Glu Ile Asp Met Gly Glu Asn Pro Gly Leu Val Thr Tyr Met Arg
                475                 480                 485

ACT CCA ATA CAA GCT GTG GTT TCT CCT AGG CTC CCC TCA CAT TTA TAC      1543
Thr Pro Ile Gln Ala Val Val Ser Pro Arg Leu Pro Ser His Leu Tyr
            490                 495                 500

AAA CGT GTG CCT GCT GAG ATC TAATCTTTCT TTTCTTTCCC TTGGACTACT         1594
Lys Arg Val Pro Ala Glu Ile
        505

CTTTGTTGCA TTAAGAAAAA TGCCTTGTGG CACTACTTTT ATCTTTGTGT TTATGTAACT    1654

ACATATGAAA TCACAATTTA AGGAACTAAG GAAAAACTCA TTGCGAGGGT              1704
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 509 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Met Ala Leu Leu Leu Ile Ile Pro Ile Ser Leu Val Thr Leu Trp Leu
  1               5                  10                  15

Gly Tyr Thr Leu Tyr Gln Arg Leu Arg Phe Lys Leu Pro Pro Gly Pro
            20                  25                  30

Arg Pro Trp Pro Val Val Gly Asn Leu Tyr Asp Ile Lys Pro Val Arg
        35                  40                  45

Phe Arg Cys Phe Ala Glu Trp Ala Gln Ser Tyr Gly Pro Ile Ile Ser
    50                  55                  60

Val Trp Phe Gly Ser Thr Leu Asn Val Ile Val Ser Asn Ser Glu Leu
65                  70                  75                  80

Ala Lys Glu Val Leu Lys Glu His Asp Gln Leu Leu Ala Asp Arg His
                85                  90                  95
```

Arg Ser Arg Ser Ala Ala Lys Phe Ser Arg Asp Gly Lys Asp Leu Ile
100 105 110

Trp Ala Asp Tyr Gly Pro His Tyr Val Lys Val Arg Lys Val Cys Thr
115 120 125

Leu Glu Leu Phe Ser Pro Lys Arg Leu Glu Ala Leu Arg Pro Ile Arg
130 135 140

Glu Asp Glu Val Thr Ser Met Val Asp Ser Val Tyr Asn His Cys Thr
145 150 155 160

Ser Thr Glu Asn Leu Gly Lys Gly Ile Leu Leu Arg Lys His Leu Gly
165 170 175

Val Val Ala Phe Asn Asn Ile Thr Arg Leu Ala Phe Gly Lys Arg Phe
180 185 190

Val Asn Ser Glu Gly Val Met Asp Glu Gln Gly Val Glu Phe Lys Ala
195 200 205

Ile Val Glu Asn Gly Leu Lys Leu Gly Ala Ser Leu Ala Met Ala Glu
210 215 220

His Ile Pro Trp Leu Arg Trp Met Phe Pro Leu Glu Glu Gly Ala Phe
225 230 235 240

Ala Lys His Gly Ala Arg Arg Asp Arg Leu Thr Arg Ala Ile Met Ala
245 250 255

Glu His Thr Glu Ala Arg Lys Lys Ser Gly Gly Ala Lys Gln His Phe
260 265 270

Val Asp Ala Leu Leu Thr Leu Gln Asp Lys Tyr Asp Leu Ser Glu Asp
275 280 285

Thr Ile Ile Gly Leu Leu Trp Asp Met Ile Thr Ala Gly Met Asp Thr
290 295 300

Thr Ala Ile Ser Val Glu Trp Ala Met Ala Glu Leu Ile Arg Asn Pro
305 310 315 320

Arg Val Gln Gln Lys Val Gln Glu Glu Leu Asp Arg Val Ile Gly Leu
325 330 335

Glu Arg Val Met Thr Glu Ala Asp Phe Ser Asn Leu Pro Tyr Leu Gln
340 345 350

Cys Val Thr Lys Glu Ala Met Arg Leu His Pro Pro Thr Pro Leu Met
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Leu Pro His Arg Ala Asn Ala Asn Val Lys Val Gly Gly Tyr Asp Ile
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Pro Lys Gly Ser Asn Val His Val Asn Val Trp Ala Val Ala Arg Asp
385 390 395 400

Pro Ala Val Trp Lys Asp Pro Leu Glu Phe Arg Pro Glu Arg Phe Leu
405 410 415

```
Glu Glu Asp Val Asp Met Lys Gly His Asp Phe Arg Leu Leu Pro Phe
            420                 425                 430

Gly Ser Gly Arg Arg Val Cys Pro Gly Ala Gln Leu Gly Ile Asn Leu
        435                 440                 445

Ala Ala Ser Met Leu Gly His Leu Leu His His Phe Cys Trp Thr Pro
    450                 455                 460

Pro Glu Gly Met Lys Pro Glu Glu Ile Asp Met Gly Glu Asn Pro Gly
465                 470                 475                 480

Leu Val Thr Tyr Met Arg Thr Pro Ile Gln Ala Val Val Ser Pro Arg
                485                 490                 495

Leu Pro Ser His Leu Tyr Lys Arg Val Pro Ala Glu Ile
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(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGTCTAACTC CTTCCTTTTC 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

```
Phe Leu Pro Phe Gly Xaa Gly Xaa Arg Xaa Cys Xaa Gly
1               5                   10
```

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Phe Xaa Xaa Gly Xaa Xaa Xaa Cys Xaa Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Xaa Cys Xaa Gly
1

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Pro Glu Glu Phe Xaa Pro Glu Arg Phe
1 5

THAT WHICH IS CLAIMED IS:

1. An isolated DNA molecule comprising a sequence selected from the group consisting of:

a) SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, and SEQ ID NO:17;

b) DNA sequences which encode an enzyme having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO6:, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, and SEQ ID NO:18;

c) DNA sequences which have at least about 90% sequence identity to the DNA of (a) or (b) above and which encode a cytochrome P450 enzyme; and

d) DNA sequences which differ from the DNA of (a) or (c) above due to the degeneracy of the genetic code.

2. A peptide encoded by a DNA sequence of claim 1.

3. A cytochrome p450 enzyme having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO6:, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, and SEQ ID NO:18.

4. An isolated DNA molecule comprising a sequence selected from the group consisting of:

a) SEQ ID NO:1;

b) DNA sequences which encode an enzyme having SEQ ID NO:2,;

c) DNA sequences which have at least about 90% sequence identity to the DNA of (a) or (b) above and which encode a cytochrome P450 enzyme; and

d) DNA sequences which differ from the DNA of (a) or (c) above due to the degeneracy of the genetic code.

5. A peptide encoded by a DNA sequence of claim 4.

6. A cytochrome p450 peptide having SEQ ID NO:2.

7. A DNA construct comprising an expression cassette, which construct comprising in the 5' to 3' direction, a promoter operable in a plant cell and a DNA segment according to claim 1 positioned downstream from said promoter and operatively associated therewith.

8. A DNA construct according to claim 7, wherein said promoter is constitutively active in plant cells.

9. A DNA construct according to claim 7, wherein said promoter is the 35S promoter from Cauliflower Mosaic virus.

10. A DNA construct according to claim 7, said construct further comprising a plasmid.

11. A DNA construct according to claim 7 carried by a plant transformation vector.

12. A DNA construct according to claim 7 carried by an *Agrobacterium tumefaciens* plant transformation vector.

13. A plant cell containing a DNA construct according to claim 7.

14. A transgenic plant comprising plant cells according to claim 13.

15. A transgenic plant according to claim 14, wherein said plant is a monocot.

16. A transgenic plant according to claim 14, wherein said plant is a dicot.

17. A DNA construct comprising an expression cassette, which construct comprising in the 5' to 3' direction, a promoter operable in a plant cell, and a DNA segment encoding a peptide of SEQ ID NO:2 positioned downstream from said promoter and operatively associated therewith.

18. A DNA construct according to claim 17, wherein said promoter is constitutively active in plant cells.

19. A DNA construct according to claim 17, wherein said promoter is the 35S promoter from Cauliflower Mosaic virus.

20. A DNA construct according to claim 17, said construct further comprising a plasmid.

21. A DNA construct according to claim 17 carried by a plant transformation vector.

22. A DNA construct according to claim 17 carried by an *Agrobacterium tumefaciens* plant transformation vector.

23. A plant cell containing a DNA construct according to claim 17.

24. A transgenic plant comprising plant cells according to claim 23.

25. A transgenic plant according to claim 24, wherein said plant is a monocot.

26. A transgenic plant according to claim 24, wherein said plant is a dicot.

27. A method of making a transgenic plant cell having an increased ability to metabolize phenylurea compounds compared to an untransformed plant cell, said method comprising:

a) providing a plant cell;

b) transforming said plant cell with an exogenous DNA construct comprising, in the 5' to 3' direction, a promoter operable in a plant cell and a DNA sequence encoding a peptide of SEQ ID NO:2, said DNA sequence operably linked to said promoter.

28. A method according to claim 27, wherein said plant cell is from a member of the Solanacae family.

29. A method according to claim 27, wherein said promoter is the 35S promoter from Cauliflower Mosaic virus.

30. A method according to claim 27, wherein said transforming step is carried out by bombarding said plant cell with microparticles carrying said DNA construct.

31. A method according to claim 27 wherein said transforming step is carried out by infecting said plant cell with an *Agrobacterium tumefaciens* containing a Ti plasmid carrying said DNA construct.

32. A method according to claim 27, further comprising regenerating a plant from said transformed plant cell.

33. A transformed plant produced by the method of claim 32.

34. Seed or progeny of a plant according to claim 33, which seed or progeny has inherited said DNA sequence encoding a peptide of SEQ ID NO:2.

35. A transformed plant produced by the method of claim 32, which plant has increased resistance to phenylurea herbicides compared to wild-type plants of the same species.

36. A transgenic plant having an increased ability to metabolize phenylurea compounds compared to an untransformed plant cell, said transgenic plant comprising transgenic plant cells containing an exogenous DNA construct comprising, in the 5' to 3' direction, a promoter operable in said plant cell, said promoter operably linked to a DNA sequence encoding a peptide of SEQ ID NO:2.

37. A transgenic plant according to claim 36, wherein said promoter is the 35S promoter from Cauliflower Mosaic virus.

38. A transgenic plant according to claim 36, wherein said plant is a dicot.

39. A transgenic plant according to claim 36, wherein said plant is a monocot.

40. A transgenic plant according to claim 36, wherein said plant is a member of the family Solanacae.

41. A transgenic plant according to claim 36, which plant is selected from the group consisting of tobacco, potato, tomato, corn, rice, cotton, soybean,

rape, wheat, oats, barley, rye and rice.

42. Progeny or seed of a plant according to claim 36, wherein said seed or progeny has inherited said DNA sequence encoding a peptide of SEQ ID NO:2.

43. A transformed plant according to claim 36, which plant has increased resistance to phenylurea herbicides compared to wild-type plants of the same species.

44. A crop comprising a plurality of plants according to claim 36 planted in an agricultural field.

45. A method of using a phenylurea herbicide as a post-emergence herbicide, comprising:

a) planting a crop according to claim 44;

b) applying to said crop a phenylurea herbicide.

46. A method according to claim 45, wherein said crop is selected from the group consisting of turfgrass, tobacco, potato, tomato, corn, rice, cotton, soybean, rape, wheat, oats, barley, rye and rice.

47. A method according to claim 45, wherein said herbicide is selected from the group consisting of fluometuron, linuron, chlortoluron and diuron.

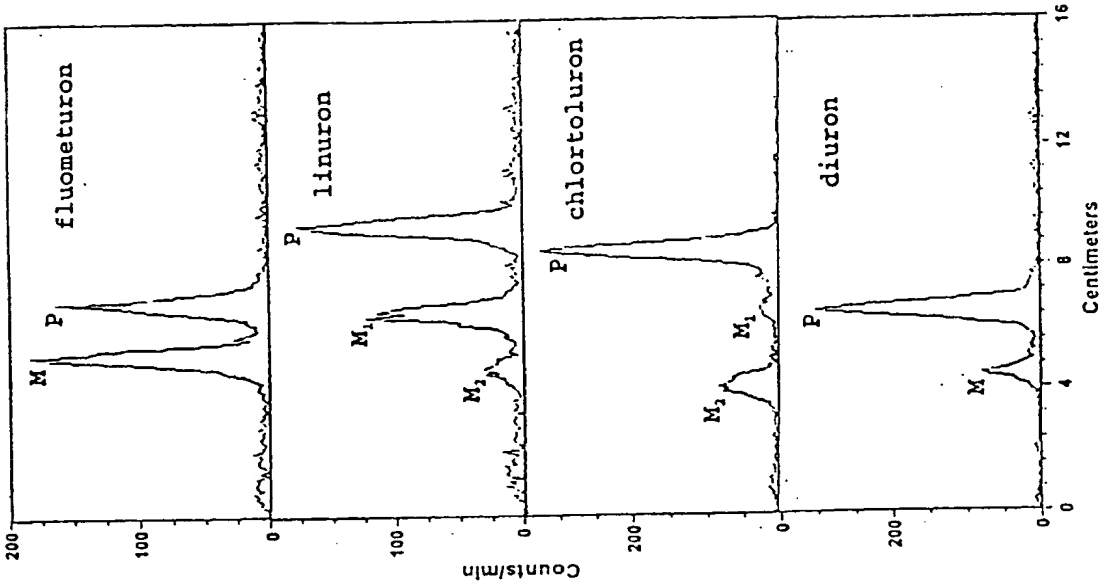

Fig. 2

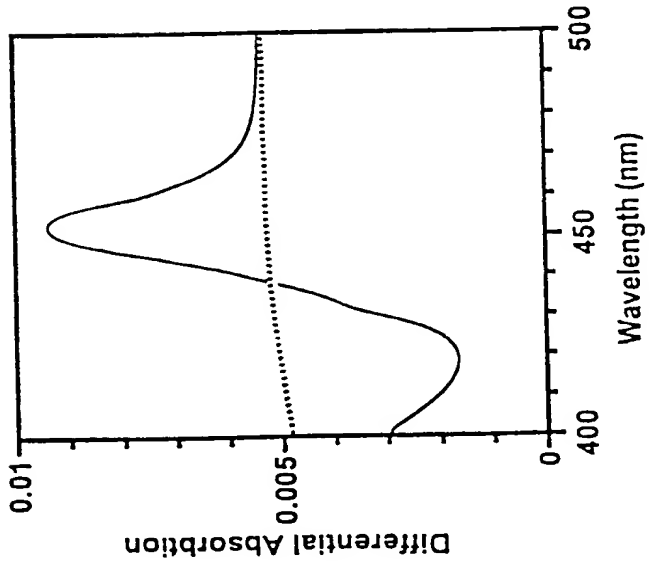

Fig. 1

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(57) Abstract

DNA sequence encoding novel cytochrome P-450 molecules are provided. The use of DNA constructs containing such molecules to transform plants is described, as are transgenic plants exhibiting increased resistance to phenylurea herbicides. Methods of using such DNA constructs and transformed plants are provided.

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CYTOCHROME P-450 CONSTRUCTS AND METHODS OF PRODUCING HERBICIDE-RESISTANT TRANSGENIC PLANTS

Field of the Invention

The present invention relates to DNA encoding novel cytochrome P-450 molecules, and the transformation of cells with such DNA. These DNA sequences may be used in methods of producing plants with an altered ability to metabolize chemical compounds, such as phenylurea herbicides.

Background of the Invention

Cytochrome P-450 (P-450) monooxygenases are ubiquitous hemoproteins present in microorganisms, plants and animals. Comprised of a large and diverse group of isozymes, P-450s mediate a great array of oxidative reactions using a wide range of compounds as substrates, and including biosynthetic processes such as phenylpropanoid, fatty acid, and terpenoid biosynthesis; metabolism of natural products; and detoxification of foreign substances (xenobiotics). *See e.g.*, Schuler, *Crit. Rev. Plant Sci.* 15:235-284 (1996). In a typical P-450 catalyzed reaction, one atom of molecular oxygen (O_2) is incorporated into the substrate, and the other atom is reduced to water by NADPH. For most eucaryotic P-450s, NADPH:cytochrome P-450 reductase, a membrane-bound flavoprotein, transfers the necessary two electrons from NADPH to the P-450 (Bolwell et al, *Phytochemistry* 37: 1491-1506 (1994)).

Frear et al. (Phytochemistry 8:2157-2169 (1969)) demonstrated the metabolism of monuron by a mixed-function oxidase located in a microsomal fraction of cotton seedlings. Further evidence has accumulated supporting the involvement of P-450s in the metabolism and detoxification of numerous herbicides representing several distinct classes of compounds (reviewed in Bolwell et al., 1994; Schuler, 1996). Differential herbicide metabolizing P-450 activities are believed to represent one of the mechanisms that enables certain crop species to be more tolerant of a particular herbicide than other crop or weedy species.

Summary of the Invention

A first aspect of the present invention is an isolated DNA molecule comprising SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, or SEQ ID NO:17; or DNA sequences which encode an enzyme of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO6:, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18; or DNA sequences which have at least about 90% sequence identity to the above DNA and which encode a cytochrome P450 enzyme; and DNA sequences which differ from the above DNA due to the degeneracy of the genetic code.

A further aspect of the present invention is a cytochrome p450 enzyme having an amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO6:, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.

A further aspect of the present invention is an isolated DNA molecule comprising SEQ ID NO:1; DNA sequences which encode an enzyme of SEQ ID NO:2,; DNA sequences which have at least about 90% sequence identity to the above DNA and which encode a cytochrome P450 enzyme; and DNA sequences which differ from the above DNA due to the degeneracy of the genetic code.

A further aspect of the present invention is a cytochrome p450 peptide of SEQ ID NO:2.

A further aspect of the present invention is a DNA construct comprising a promoter operable in a plant cell and a DNA segment encoding a peptide of SEQ ID NO:2 downstream from and operatively associated with the promoter.

A further aspect of the present invention is a method of making a transgenic plant cell having an increased ability to metabolize phenylurea compounds compared to an untransformed plant cell. The plant cell is transformed with an exogenous DNA construct comprising a promoter operable in a plant cell and a DNA sequence encoding a peptide of SEQ ID NO:2. Transformed plants, seed and progeny of such plants are also aspects of the

present invention.

A further aspect of the present invention is a transgenic plant having an increased ability to metabolize phenylurea compounds. Such transgenic plants contain exogenous DNA encoding a peptide of SEQ ID NO:2.

Brief Description of the Drawings

Figure 1 depicts dithionite-reduced carbon monoxide difference spectra, where the solid line represents microsomes isolated from yeast transformed with CYP71A10, and the dotted line shows the difference spectra from yeast transformed with control vector V-60. Microsomal protein concentration was 1 mg/ml.

Figure 2 shows thin-layer chromatograms of [^{14}C]-radiolabeled fluometuron, linuron, chlortoluron, and diuron and their respective metabolites after incubation of the radiolabeled herbicides with yeast microsomes containing the CYP71A10 protein. Initial substrate concentrations for fluometuron, linuron, chlortoluron and diuron were 5.2, 6.5, 4.0, and 3.7 μM, respectively. P = parent compound; M = metabolite.

Figure 3 shows the chemical structures of fluometuron, linuron, chlortoluron and diuron, and their previously characterized metabolites. The linuron and chlortoluron metabolites are designated major or minor depending on their predicted relative abundance in assays using yeast microsomes containing the soybean CYP71A10 protein.

Figure 4 shows thin-layer chromatograms using [^{14}C]-radiolabeled linuron in various control reactions. The complete reaction mixture (COMPLETE) contained 3.2 μM linuron, 0.75 mM NADPH and 2.5 mg/ml microsomal protein isolated from CYP71A10-transformed yeast in 50 mM phosphate buffer (pH 7.1). Other reactions varied from COMPLETE by the addition of carbon monoxide (+CO), the omission of NADPH (NO NADPH), or the use of yeast microsomes isolated from cells expressing the control vector (V-60). P = parent compound; M = metabolite.

Figure 5A shows tobacco line 25/2 plants (transformed with soybean CYP71A10) grown on media containing no herbicide.

Figure 5B shows control tobacco plants (transformed with vector pBI121) grown on media containing 0.5 μM linuron.

Figure 5C shows tobacco line 25/2 (transformed with soybean CYP71A10) individuals grown on media containing 0.5 μM linuron.

Figure 5D shows tobacco line 25/2 (transformed with soybean CYP71A10) individuals grown on media containing 2.5 μM linuron.

Figure 5E shows control tobacco plants (transformed with vector pBI121) grown on media containing 1.0 μM chlortoluron.

Figure 5F shows tobacco line 25/2 (transformed with soybean CYP71A10) individuals grown on media containing 1.0 μM chlortoluron.

Detailed Description of the Invention

1. Overview of the present research:

The present inventors utilized a strategy based on the random isolation and screening of soybean cDNAs encoding cytochrome P-450 (P-450) isozymes to identify P-450 isozymes involved in herbicide metabolism. Eight full-length and one near full-length P-450 cDNAs representing eight distinct P-450 families were isolated using polymerase chain reaction (PCR)-based technologies (**SEQ ID NOS:1, 3, 5, 7, 9, 11, 13, 15** and 17). Five of these soybean P-450 cDNAs were successfully overexpressed in yeast, and microsomal fractions generated from these strains were tested for their potential to mediate the metabolism of ten herbicides and one insecticide. *In vitro* enzyme assays showed that the gene product of one heterologously expressed P-450 cDNA (CYP71A10) (**SEQ ID NO:1**) specifically mediated the metabolism of phenylurea herbicides, converting four herbicides of this class (fluometuron, linuron, chlortoluron, and diuron) into more polar metabolites. Analyses of the metabolites indicate that the CYP71A10 encoded enzyme functions primarily as an N-demethylase with regard to

fluometuron, linuron and diuron, and as a ring-methyl hydroxylase when chlortoluron is the substrate. *In vivo* assays using excised leaves demonstrated that all four herbicides were more readily metabolized in CYP71A10-transformed tobacco in comparison to control plants.

Shiota et al. reported that fused constructs derived from the rat CYP1A1 and yeast NADPH-cytochrome P-450 oxidoreductase cDNAs conferred chlortoluron resistance in tobacco by enhancing herbicide metabolism (Shiota et al., *Plant Physiol.* 106:17-23 (1994)). In another study, a chloroplast-targeted, bacterial CYP105A1 expressed in tobacco catalyzed the toxification of R7402, a sulfonylurea pro-herbicide (O'Keefe et al., *Plant Physiol.* 105:473-482 (1994)). The cloning and heterologous expression of an endogenous plant P-450 gene that is potentially involved in herbicide metabolism was reported by Pierrel et al., *Eur. J. Biochem.* 224:835-844 (1994), where a trans-cinnamic acid hydroxylase cDNA (CYP73A1) isolated from artichoke and expressed in yeast catalyzed the ring-methyl hydroxylation of chlortoluron. *In vivo* experiments with artichoke tubers, however, demonstrated that the ring-methyl hydroxy metabolite represented only a minor portion of the metabolites produced and that the major metabolite was demethylated chlortoluron (Pierrel et al., 1994). This together with the observation that the turnover number of the heterologously expressed enzyme was very low (0.014/ min), suggested that CYP73A1 plays a minimal role in chlortoluron metabolism *in vivo*. US Patent No. 5,349,127 to Dean et al. discloses the use of DNA encoding certain P-450 enzymes, isolated from *Streptomyces griseolus*, to produce transformed plants with increased metabolism of certain compounds. (All US patents referred to herein are intended to be incorporated herein in their entirety.)

Although the role of P-450 enzymes in catalyzing the metabolism of a variety of herbicides has been documented, little progress has been made in the identification of the endogenous plant P-450s that are responsible for degrading these compounds. Protein purification of specific isozymes involved in the metabolism of a specific herbicide has been hindered by the instability of the

enzymes, their low concentrations in most plant tissues, and difficulties in the reconstitution of active complexes from solubilized components. Furthermore, any given plant tissue may possess dozens, if not hundreds, of unique P-450 isozymes, complicating the purification to homogeneity of a particular isozyme. Because plants have only been exposed to phenylurea herbicides during the past few decades, it is unlikely that enzymes have evolved solely for the purposed of metabolizing this class of xenobiotics.

2. Use of CYP71A10 to produce phenylurea-resistant plants:

The present invention provides materials and methods useful in producing transgenic plant cells and plants with increased resistance to phenylurea herbicides. Increased herbicide resistance, as used herein, refers to the ability of a plant to withstand levels of an herbicide that have a negative impact on wild-type (untransformed) plants of the same species and/or variety. Resistance, as used herein, does not necessarily mean that the resistant plant is completely unaffected by exposure to the herbicide; rather, resistant plants suffer less extensive or less severe damage than comparable wild-type plants. Methods of assessing the extent and/or severity of herbicide impact will vary depending on the particular plant and the particular herbicide being tested; such assessment methods will be apparent to those skilled in the art. The negative effects of a herbicide may be evidenced by the complete arrest of plant growth, or by an inhibition in the rate or amount of growth. Additionally, methods of the present invention may be used to decrease herbicide residues in plants, even where the amounts of herbicides present in the plant do not cause an appreciable negative effect on the plant as a whole.

Increased resistance to a herbicide can be due to an increased ability to metabolize a herbicide to less harmful metabolites. Accordingly, plants of the present invention which exhibit increased resistance to a herbicide may also be described as having an increased ability to metabolize the starting herbicidal compound, where the metabolites are less harmful to the plant than the starting

compound.

In the examples provided herein, yeast microsomes and transgenic tobacco plants expressing the CYP71A10 peptide (**SEQ ID NO:2**) and exposed to various phenylurea herbicides produced the same degradation products that have previously been observed when these same compounds have been incubated with metabolically active plant microsomes. These results indicate that the CYP71A10 peptide plays a role in the effective metabolism of phenylurea herbicides.

The present examples demonstrate that the overexpression of a CYP71A10 peptide of **SEQ ID NO:2** in tobacco enhanced the plant's capacity to metabolize all four phenylurea herbicides tested, and that appreciable levels of tolerance were conferred to linuron and chlortoluron. Fluometuron was the most actively metabolized compound in both the yeast and transgenic plant systems, yet the enhancement in tolerance to this herbicide at the whole plant level was not as great as for linuron and chlortoluron. While not wishing to be held to a single theory, the present inventors surmise that the lack of correlation between the rate of herbicide metabolism and herbicide tolerance may be explained by the differential toxicities of the various phenylurea derivatives produced in the CYP71A10-transformed tobacco. Consistent with this hypothesis are the previous observations that N-demethyl derivatives of fluometuron, diuron and chlortoluron are only moderately less toxic than their parent compounds (Rubin and Eshel, *Weed Sci.* 19:592-594 (1971); Dalton et al., *Weeds* 14:31-33 (1966); Ryan and Owen, *Proc. Brit. Crop Prot. Conf. Weeds* 1:317-324 (1982)). In contrast, linuron is a 10-fold greater inhibitor of the Hill-reaction than N-demethyl linuron (Suzuki and Casida, *J. Agric. Food Chem.* 29:1027-1033 (1981)), and the hydroxylated and the didemethlayed derivatives of chlortoluron are considered to be nonherbicidal (Ryan and Owen, 1982).

The present inventors found that the relative rates of herbicide metabolism in leaves of CYP71A10-transformed tobacco and in yeast microsomes assayed *in vitro* were similar (see **Tables 4 and 5**). With the exception of the transgenic

plant leaves showing a somewhat greater metabolic activity against chlortoluron than was apparent in the yeast microsomal assays, both systems followed the general order of metabolism of fluometuron $\geq$ linuron > chlortoluron > diuron. These results indicate that expression of a test plant P-450 in yeast and quantification of the metabolism of a test compound using yeast microsomes, is a suitable system for screening plant P-450s for their metabolic function, and for their potential usefulness in the production of transgenic plants with altered metabolism of chemical compounds such as herbicides and insecticides.

The present inventors have shown that the random isolation of P-450 cDNAs with subsequent heterologous expression in yeast is an effective strategy to characterize cDNAs whose product is capable of affecting the metabolism of a test compound. This approach is useful in characterizing the substrates (both natural and artificial) affected by a P-450, in determining the function of P-450 genes whose catalytic activities remain unclear, and in screening P-450s for the ability to increase or decrease the metabolism of a test compound. A particularly useful aspect of this method is the ability to screen isolated P-450s for their effects on the metabolism by plants of herbicides, insecticides, or other chemical compounds. Increased metabolism may result in enhanced resistance to the effects of a compound (where the metabolites are less harmful than the starting compound), or in increased sensitivity to the effects of a compound (where one or more metabolites are more toxic than the starting compound; *see* O'Keefe et al., 1994).

3. DNA Constructs:

Those familiar with recombinant DNA methods available in the art will recognize that one can employ a cDNA molecule (or a chromosomal gene or genomic sequence) encoding a P-450 peptide, joined in the sense orientation with appropriate operably linked regulatory sequences, to construct transgenic cells and plants. (Those of skill in the art will also recognize that appropriate regulatory sequences for expression of genes in the sense orientation include any

one of the known eukaryotic translation start sequences, in addition to the promoter and polyadenylation/transcription termination sequences described herein). Appropriate selection of the encoded P-450 peptide will provide transformed plants characterized by altered (enhanced or retarded) metabolism of phenylurea compounds.

DNA constructs, or "transcription cassettes," of the present invention include, 5' to 3' in the direction of transcription, a promoter as discussed herein, a DNA sequence as discussed herein operatively associated with the promoter, and, optionally, a termination sequence including stop signal for RNA polymerase and a polyadenylation signal for polyadenylase. All of these regulatory regions should be capable of operating in the cells of the tissue to be transformed. Any suitable termination signal may be employed in carrying out the present invention, examples thereof including, but not limited to, the nopaline synthase (nos) terminator, the octapine synthase (ocs) terminator, the CaMV terminator, or native termination signals derived from the same gene as the transcriptional initiation region or derived from a different gene. See, e.g., Rezian et al. (1988) supra, and Rodermel et al. (1988), supra.

The term "operatively associated," as used herein, refers to DNA sequences on a single DNA molecule which are associated so that the function of one is affected by the other. Thus, a promoter is operatively associated with a DNA when it is capable of affecting the transcription of that DNA (i.e., the DNA is under the transcriptional control of the promoter). The promoter is said to be "upstream" from the DNA, which is in turn said to be "downstream" from the promoter.

The transcription cassette may be provided in a DNA construct which also has at least one replication system. For convenience, it is common to have a replication system functional in Escherichia coli, such as ColE1, pSC101, pACYC184, or the like. In this manner, at each stage after each manipulation, the resulting construct may be cloned, sequenced, and the correctness of the manipulation determined. In addition, or in place of the E. coli replication

system, a broad host range replication system may be employed, such as the replication systems of the P-1 incompatibility plasmids, e.g., pRK290. In addition to the replication system, there will frequently be at least one marker present, which may be useful in one or more hosts, or different markers for individual hosts. That is, one marker may be employed for selection in a prokaryotic host, while another marker may be employed for selection in a eukaryotic host, particularly the plant host. The markers may be protection against a biocide, such as antibiotics, toxins, heavy metals, or the like; may provide complementation, by imparting prototrophy to an auxotrophic host; or may provide a visible phenotype through the production of a novel compound in the plant.

The various fragments comprising the various constructs, transcription cassettes, markers, and the like may be introduced consecutively by restriction enzyme cleavage of an appropriate replication system, and insertion of the particular construct or fragment into the available site. After ligation and cloning the DNA construct may be isolated for further manipulation. All of these techniques are amply exemplified in the literature as exemplified by J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989)(Cold Spring Harbor Laboratory).

Vectors which may be used to transform plant tissue with nucleic acid constructs of the present invention include both Agrobacterium vectors and ballistic vectors, as well as vectors suitable for DNA-mediated transformation.

4. Promoters:

The term 'promoter' refers to a region of a DNA sequence that incorporates the necessary signals for the efficient expression of a coding sequence. This may include sequences to which an RNA polymerase binds but is not limited to such sequences and may include regions to which other regulatory proteins bind together with regions involved in the control of protein translation and may include coding sequences.

Promoters employed in carrying out the present invention may be constitutively active promoters. Numerous constitutively active promoters which are operable in plants are available. A preferred example is the Cauliflower Mosaic Virus (CaMV) 35S promoter which is expressed constitutively in most plant tissues. Use of the CaMV promoter for expression of recombinant genes in tobacco roots has been well described (Lam et al., "Site-Specific Mutations Alter In Vitro Factor Binding and Change Promoter Expression Pattern in Transgenic Plants", *Proc. Nat. Acad. Sci. USA* 86, pp. 7890-94 (1989); Poulsen et al. "Dissection of 5' Upstream Sequences for Selective Expression of the Nicotiana plumbaginifolia rbcS-8B Gene", *Mol. Gen. Genet.* 214, pp. 16-23 (1988)). In the alternative, the promoter may be a tissue-specific promoter or a promoter that is expressed temporally or developmentally. See, e.g., US Patent No. 5,459,252 to Conkling et al.; Yamamoto et al., *The Plant Cell*, 3:371 (1991). In methods of transforming plants to alter the effects of herbicides or to decrease residual amounts of herbicides or pesticides in plants, selection of a suitable promoter will vary depending on the plant species, the specific chemical compound used as a herbicide or pesticide, and the time and method of applying the chemical compound to the plant or plant crop, as will be apparent to those skilled in the art.

5. Selectable Markers:

The recombinant DNA molecules and vectors used to produce the transformed cells and plants of this invention may further comprise a dominant selectable marker gene. Suitable dominant selectable markers include, inter alia, antibiotic resistance genes encoding neomycin phosphotransferase (NPTII), hygromycin phosphotransferase (HPT), and chloramphenicol acetyltransferase (CAT). Another well-known dominant selectable marker suitable is a mutant dihydrofolate reductase gene that encodes methotrexate-resistant dihydrofolate reductase. DNA vectors containing suitable antibiotic resistance genes, and the corresponding antibiotics, are commercially available. Transformed cells are

selected out of the surrounding population of non-transformed cells by placing the mixed population of cells into a culture medium containing an appropriate concentration of the antibiotic (or other compound normally toxic to the untransformed cells) against which the chosen dominant selectable marker gene product confers resistance. Thus, only those cells that have been transformed will survive and multiply.

A further aspect of the present invention is use of the identified P-450 coding sequences as a selectable marker gene. A DNA construct comprising a sequence encoding a P-450 known to increase resistance to a compound (such as **SEQ ID NO:2**) is utilized to transform cells, in accordance with methods known in the art. Those cells that subsequently exhibit resistance to the compound are indicated as transformed. Such constructs may be used to verify the success of a transformation technique or to select transformed cells of interest.

6. Sequence similarity and hybridization conditions:

Nucleic acid sequences employed in carrying out the present invention include those with sequence similarity to **SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15** or **17**, and encoding a protein having P-450 enzymatic activity. This definition is intended to encompass natural allelic variants and minor sequence variations in the nucleic acid sequence encoding a P-450 molecule, or minor sequence variations in the amino acid sequence of the encoded product. Thus, DNA sequences that hybridize to DNA of **SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15** or **17** and code for expression of a P-450 enzyme, particularly a plant P-450 enzyme, may also be employed in carrying out aspects of the present invention. The nomenclature for P-450 genes is based on amino acid sequence identity; methods of determining sequence similarity are well-known to those skilled in the art. Typically, sequences sharing >40% identity are placed in the same family, >55% identity defines members of the same subfamily, and sequences that

display >97% identity are assumed to represent allelic variants. Conditions which permit other DNA sequences which code for expression of a protein having P-450 enzymatic activity to hybridize to DNA of **SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15** or **17**, or to other DNA sequences encoding the protein given as **SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16** or **18** can be determined in a routine manner. For example, hybridization of such sequences may be carried out under conditions of reduced stringency or even stringent conditions (e.g., conditions represented by a wash stringency of 0.3 M NaCl, 0.03 M sodium citrate, 0.1% SDS at 60°C or even 70°C to DNA encoding the protein given as **SEQ ID NO:2** herein in a standard in situ hybridization assay. See J. Sambrook et al., Molecular Cloning, A Laboratory Manual (2d Ed. 1989)(Cold Spring Harbor Laboratory)). In general, such sequences will be at least 65% similar, 75% similar, 80% similar, 85% similar, 90% similar, 93% similar, 95% similar, or even 97% or 98% similar, or more, with the sequence given herein as **SEQ ID NO:1**, or DNA sequences encoding proteins of **SEQ ID NO:2**. (Determinations of sequence similarity are made with the two sequences aligned for maximum matching; gaps in either of the two sequences being matched are allowed in maximizing matching. Gap lengths of 10 or less are preferred, gap lengths of 5 or less are more preferred, and gap lengths of 2 or less still more preferred.)

As used herein, the term 'gene' refers to a DNA sequence that incorporates (1) upstream (5') regulatory signals including a promoter, (2) a coding region specifying the product, protein or RNA of the gene, (3) downstream (3') regions including transcription termination and polyadenylation signals and (4) associated sequences required for efficient and specific expression.

The DNA sequence of the present invention may consist essentially of a sequence provided herein (**SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15** or **17**), or equivalent nucleotide sequences representing alleles or polymorphic variants of these genes, or coding regions thereof.

Use of the phrase "substantial sequence similarity" in the present

specification and claims means that DNA, RNA or amino acid sequences which have slight and non-consequential sequence variations from the actual sequences disclosed and claimed herein are considered to be equivalent to the sequences of the present invention. In this regard, "slight and non-consequential sequence variations" mean that "similar" sequences (i.e., the sequences that have substantial sequence similarity with the DNA, RNA, or proteins disclosed and claimed herein) will be functionally equivalent to the sequences disclosed and claimed in the present invention. Functionally equivalent sequences will function in substantially the same manner to produce substantially the same compositions as the nucleic acid and amino acid compositions disclosed and claimed herein.

DNA sequences provided herein can be transformed into a variety of host cells. A variety of suitable host cells, having desirable growth and handling properties, are readily available in the art.

Use of the phrase "isolated" or "substantially pure" in the present specification and claims as a modifier of DNA, RNA, polypeptides or proteins means that the DNA, RNA, polypeptides or proteins so designated have been separated from their *in vivo* cellular environments through the efforts of human beings.

As used herein, a "native DNA sequence" or "natural DNA sequence" means a DNA sequence which can be isolated from non-transgenic cells or tissue. Native DNA sequences are those which have not been artificially altered, such as by site-directed mutagenesis. Once native DNA sequences are identified, DNA molecules having native DNA sequences may be chemically synthesized or produced using recombinant DNA procedures as are known in the art. As used herein, a native plant DNA sequence is that which can be isolated from non-transgenic plant cells or tissue.

7. Transformed plants:

Methods of making recombinant plants of the present invention, in general, involve first providing a plant cell capable of regeneration (the plant cell

typically residing in a tissue capable of regeneration). The plant cell is then transformed with a DNA construct comprising a transcription cassette of the present invention (as described herein) and a recombinant plant is regenerated from the transformed plant cell. As explained below, the transforming step is carried out by techniques as are known in the art, including but not limited to bombarding the plant cell with microparticles carrying the transcription cassette, infecting the cell with an Agrobacterium tumefaciens containing a Ti plasmid carrying the transcription cassette, or any other technique suitable for the production of a transgenic plant.

Numerous Agrobacterium vector systems useful in carrying out the present invention are known. For example, U.S. Patent No. 4,459,355 discloses a method for transforming susceptible plants, including dicots, with an Agrobacterium strain containing the Ti plasmid. The transformation of woody plants with an Agrobacterium vector is disclosed in U.S. Patent No. 4,795,855. Further, U.S. Patent No. 4,940,838 to Schilperoort et al. discloses a binary Agrobacterium vector (i.e., one in which the Agrobacterium contains one plasmid having the vir region of a Ti plasmid but no T region, and a second plasmid having a T region but no vir region) useful in carrying out the present invention.

Microparticles carrying a DNA construct of the present invention, which microparticle is suitable for the ballistic transformation of a plant cell, are also useful for making transformed plants of the present invention. The microparticle is propelled into a plant cell to produce a transformed plant cell, and a plant is regenerated from the transformed plant cell. Any suitable ballistic cell transformation methodology and apparatus can be used in practicing the present invention. Exemplary apparatus and procedures are disclosed in Sanford and Wolf, U.S. Patent No. 4,945,050, and in Christou et al., U.S. Patent No. 5,015,580. When using ballistic transformation procedures, the transcription cassette may be incorporated into a plasmid capable of replicating in or integrating into the cell to be transformed. Examples of microparticles suitable

for use in such systems include 1 to 5 μm gold spheres. The DNA construct may be deposited on the microparticle by any suitable technique, such as by precipitation.

Plant species may be transformed with the DNA construct of the present invention by the DNA-mediated transformation of plant cell protoplasts and subsequent regeneration of the plant from the transformed protoplasts in accordance with procedures well known in the art. Fusion of tobacco protoplasts with DNA-containing liposomes or via electroporation is known in the art. (Shillito et al., "Direct Gene Transfer to Protoplasts of Dicotyledonous and Monocotyledonous Plants by a Number of Methods, Including Electroporation", *Methods in Enzymology* 153, pp. 313-36 (1987)).

As used herein, transformation refers to the introduction of exogenous DNA into cells, so as to produce transgenic cells stably transformed with the exogenous DNA. Transformed plant cells are induced to regenerate intact plants through application of cell and tissue culture techniques that are well known in the art. The method of plant regeneration is chosen so as to be compatible with the method of transformation. The stable presence and the orientation of the exogenous DNA in transgenic plants can be verified by Mendelian inheritance of the DNA sequence, as revealed by standard methods of DNA analysis applied to progeny resulting from controlled crosses.

Plants of horticultural or agronomic utility, such as vegetable or other crops, can be transformed according to the present invention using techniques available in the art. A plant suitable for use in the present methods is *Nicotiana tabacum*, or tobacco. Any strain or variety of tobacco may be used. Additional plants (both monocots and dicots) which may be employed in practicing the present invention include, but are not limited to, potato (*Solanum tuberosum*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus spp.*)cassava (*Manihot esculenta*), coffee (*Cofea* spp.), pineapple (*Ananas comosus*), citrus trees (*Citrus*

spp.), banana (*Musa* spp.), corn (*Zea mays*), oilseed rape (*Brassica napus*), wheat, oats, barley, rye and rice. Thus, an illustrative category of plants which may be used to practice aspects of the present invention are the dicots, and a more particular category of plants which may be used to practice the present invention are members of the family Solanacae.

The methods of the present invention can further be practiced with turfgrass, including cool season turfgrasses and warm season turfgrasses. Examples of cool season turfgrasses are Bluegrasses (*Poa* L.), such as Kentucky Bluegrass (*Poa pratensis* L.), rough Bluegrass (*Poa trivialis* L.), Canada Bluegrass (*Poa compressa* L.), Annual Bluegrass (*Poa annua* L.), Upland Bluegrass (*Poa glaucantha* Gaudin), Wood Bluegrass (*Poa nemoralis* L.), and Bulbous Bluegrass (*Poa bulbosa* L.); the Bentgrasses and Redtop (*Agrostis* L.), such as Creeping Bentgrass (*Agrostis palustris* Huds.), Colonial Bentgrass (*Agrostis tenius* Sibth.), Velvet Bentgrass (*Agrostis canina* L.), South German Mixed Bentgrass (*Agrostis* L.), and Redtop (*Agrostis alba* L.); the Fescues (*Festuca* L.), such as Red Fescue (*Festuca rubra* L.), Chewings Fescue (*Festuca rubra* var. *commutata* Gaud.), Sheep Fescue (*Festuca ovina* L.), Hard Fescue (*Festuca ovina* var. *duriuscula* L. Koch), Hair Fescue (*Festuca capillata* Lam.), Tall Fescue (*Festuca arundinacea* Schreb.), Meadow Fescue (*Festuca elatior* L.); the Rye grasses (*Lolium* L.), such as Perennial Ryegrass (*Lolium perenne* L.), Italian Ryegrass (*Lolium multiflorum* Lam.); the Wheatgrasses (*Agropyron* Gaertn.), such as Fairway Wheatgrass (*Agropyron cristatum* L. Gaertn.), Western Wheatgrass (*Agropyron smithii* Rydb.). Examples of warm season turfgrasses are the Bermudagrasses (*Cynodon* L.C. Rich), the Zoysiagrasses (*Zoysia* Willd.), St. Augustinegrasses (*Stenotaphrum secundatum* (Walt.) Kuntze), Centipedegrass (*Eremochioa ophiuroides* (Munro.) Hack.), Carpetgrass (*Axonopus* Beauv.), Bahiagrass (*Paspalum notatum* Flugge.), Kikuyugrass (*Pennisetum clandestinum* Hochst. ex Chiov.), Buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.), Blue Grama (*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.), Sideoats Grama (*Bouteloua curtipendula* (Michx.) Torr.), and Dichondra

(*Dichondra* Forst.).

Any plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a vector of the present invention. The term "organogenesis," as used herein, means a process by which shoots and roots are developed sequentially from meristematic centers; the term "embryogenesis," as used herein, means a process by which shoots and roots develop together in a concerted fashion (not sequentially), whether from somatic cells or gametes. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, callus tissue, existing meristematic tissue (e.g., apical meristems, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem).

Plants of the present invention may take a variety of forms. The plants may be chimeras of transformed cells and non-transformed cells; the plants may be clonal transformants (e.g., all cells transformed to contain the transcription cassette); the plants may comprise grafts of transformed and untransformed tissues (e.g., a transformed root stock grafted to an untransformed scion in citrus species). The transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, first generation (or T1) transformed plants may be selfed to provide homozygous second generation (or T2) transformed plants, and the T2 plants further propagated through classical breeding techniques. A dominant selectable marker (such as nptII) can be associated with the transcription cassette to assist in breeding.

As used herein, a crop comprises a plurality of plants of the same genus or species, planted together in an agricultural field. By "agricultural field" is meant a common plot of soil or a greenhouse. Thus, the present invention provides a method of producing a crop of plants having altered metabolism of chemical compounds (such as a phenylurea herbicide), and thus having altered

resistance to the chemical compound, compared to a crop of non-transformed plants of the same genus or species, or variety.

Where a crop comprises a plurality of transgenic plants with increased resistance to phenylurea compounds according to the present invention, such compounds may be used as post-emergent herbicides to control undesirable plant species. Accordingly, a method of using phenylurea compounds as post-emergent herbicides according to the present invention comprises planting a plurality of transformed plant seed (or transformed plants) with enhanced resistance to a phenylurea herbicide, and applying that herbicide to the field after the germination and emergence of at least some of said transformed plant seed (or following the planting of transformed plants). Application of the phenylurea herbicide will selectively impact non-resistant plants.

9. Microbial decontamination:

Microbial cells useful for degrading phenylurea compounds, which cells contain and express a heterologous DNA molecule encoding a P-450 enzyme that enhances the metabolism of the phenylurea compound in the microbial cell (*e.g.*, a peptide of **SEQ ID NO:2**), are a further aspect of the present invention. Suitable host microbial cells include soil microbes (*i.e.*, those which grow in the soil) transformed to express a P-450 enzyme that enhances the metabolism of one or more phenylurea compounds by the host cell. Suitable microbes include bacteria (such as *Agrobacterium, Bacillus, Streptomyces, Nocardia,* etc.), fungi (including yeasts), and algae. Microbes can be selected, by methods known in the art of soil microbiology, to correspond to those which are typically found in the substrate to be treated. Liquids which are contaminated with phenylurea compounds may be contacted to transformed microorganisms by passing the contaminated liquid through a bioreactor which contains the microorganism. Numerous suitable bioreactor designs are known in the art. A microbial host particularly suitable for bioreactors is yeast.

Combination treatments utilizing aspects of the present invention involve

the application of a phenylurea compound in a location such as an agricultural field (*e.g.*, as a herbicide), and subsequent application of a transformed microbe as described above in an amount effective to degrade residual applied herbicide. Application of the herbicide may be carried out in accordance with known techniques.

The examples which follow are set forth to illustrate the present invention, and are not to be construed as limiting thereof.

EXAMPLE 1

Materials and Methods

a. Substrates

Phenyl-U-[^{14}C] fluometuron, phenyl-U-[^{14}C] chlortoluron, phenyl-U-[^{14}C] metolachlor, phenyl-U-[^{14}C] prosulfuron, pyrimidinyl-2- diazinon, and phenyl-U-[^{14}C] alachlor were provided by Novartis (Greensboro, North Carolina); phenyl-U-[^{14}C] bentazon was donated by BASF (Research Triangle Park, North Carolina); phenyl-U-[^{14}C] linuron, phenyl-U-[^{14}C] diuron, and carbonyl-[^{14}C] metribuzin were a gift from DuPont de Nemours (Wilmington, Delaware); carboxyl-[^{14}C] imazaquin was provided by American Cyanamid (Princeton, New Jersey).

b. Isolation of P-450 cDNAs

Random amplification of partial cDNAs encoding P-450 enzymes was conducted essentially as described by Meijer et al., *Plant Mol. Biol.* 22:379-383 (1993), using a soybean (*Glycine max* cv Dare) leaf cDNA library as the template (Dewey et al., *Plant Cell* 6:1495-1507 (1994)). Briefly, degenerate inosine-containing primers were synthesized based on the highly conserved heme-binding region. The precise sequences of these primers are described in Meijer et al. (1993). An oligo-dT primer complementary to the poly(A) tail of the cDNA clones was used in conjunction with the degenerate primers in PCR amplification assays. Amplification products were cloned into the T-tailed pCRII plasmid

(Invitrogen, San Diego, CA) and DNA sequence analysis of the first 300-400 base pairs downstream of the conserved region was used to establish whether a given amplification product represented a true P-450 cDNA.

To recover full-length versions of the partial cDNAs, a primer (5'-TGTCTAACTCCTTCCTTTTC-3') (**SEQ ID NO:19**) complementary to the pYES2 vector (the vector into which the soybean cDNA library was cloned) and a downstream primer corresponding to a segment of the 3' untranslated region for each of the unique P-450 cDNAs were used in PCR reactions using the same soybean cDNA library as the template. PCR products were again cloned into the pCRII plasmid and the entire DNA sequence was determined for the largest cDNA amplified for each unique soybean P-450.

To isolate full-length versions of the respective P-450 ORFs without including any of the 5' untranslated region (which has been shown to potentially impede gene expression in yeast (Pompon, *Eur. J. Biochem.* 177:285-293 (1988)), an additional PCR reaction was performed with two gene-specific primers. The forward primers contained a BamHI restriction site immediately followed by the ATG start codon, and the next 14-15 bases of the reading frame; the downstream primer was again specific for the 3' untranslated regions of the respective genes and included sequences specifying either EcoRI, KpnI, and SacI to facilitate subcloning of the P-450 cDNAs into the yeast expression vector, pYeDP60 (V-60; Urban et al., *Biochimie* 72:463-472 (1990)).

All PCR reactions, with the exception of the initial amplification of the partial P-450 cDNAs (see Meijer et al. (1993)), contained 0.2 ng/µl template, 2 µM of each primer, 200 µM of each dNTP, and 1.5 mM $MgCl_2$ in a final reaction volume of 50 µl. Amplification was initiated by the addition of 1.5 U EXPAND™ High Fidelity enzyme mix using conditions described by the manufacturer (Boeringer Mannheim). DNA sequence was determined by the chain termination method (Sanger et al., *Proc. Natl. Acad. Sci. USA* 74:5463-5467 (1977)) using fluorescent dyes (Applied Biosystems, Foster City, CA). DNA and predicted amino acid sequences were analyzed using the BLAST

algorithm and the GAP program (University of Wisconsin, Madison, Genetics Computing Group software package).

c. P-450 cDNA Expression in Yeast

Yeast transformation was performed as described by Geitz et al., *Nucleic Acids Research* 20:1425 (1992). Media composition, culturing conditions, galactose induction, and microsomal preparations were conducted according to Pompon et al., *Methods Enzymol.* 272:51-64 (1995), using a culture volume of 250 ml. Microsomal protein was quantified spectrophotometrically using the method of Waddell, *J. Lab. Clin. Med.* 48:311-314 (1956), using bovine albumin as a standard. Dithionite-reduced, carbon monoxide difference spectra was obtained as previously outlined (Estabrook and Werringloer, *Methods Enzymol.* 52:212-220 (1978)) using a Shimadzu Recording Spectrophotometer UV-240 (Shimadzu, Kyoto, Japan). P-450 protein concentrations of yeast microsomes were calculated using a millimolar extinction coefficient of 91 (Omura and Sato, *J. Biol. Chem.*, 239:2370-2378 (1964)).

d. *In vitro* Herbicide Metabolism Assays

Yeast microsomes enriched for a discrete soybean P-450 isozyme were assayed for their capacity to metabolize the ten herbicides and one insecticide listed in Table 3. The reaction mixtures contained 10,000 DPM (100-200 ng) radiolabeled substrate, 0.75 mM NAPDH, 2.5 mg/ml microsomal protein. Total reaction volumes were adjusted to 150 µl with 50 mM phosphate buffer (pH 7.1). The mixtures were incubated under light for 45 minutes at 27°C, arrested with 50 µl acetone and centrifuged at 14 000xg for 2 minutes. Fifty microliters of the supernatants containing radiolabeled alachlor, metolachlor, metribuzin, prosulfuron, chlortoluron, diuron, fluometuron, linuron, or diazinon were spotted onto 250 micron Whatman K6F silica plates. Radiolabeled bentazon and imazaquin-containing samples were spotted onto 200 micron Whatman LKC18F silica gel reversed-phase plates. All plates were developed in a benzene/acetone

2:1 (v/v) solvent system with the exception of prosulfuron, developed in toluene/acetone/acetic acid, 75:20:5 (v/v/v), and bentazon and imazaquin, developed in methanol/75 mM sodium acetate 40:60 (v/v). The developed plates were scanned with a Bioscan System 400 imaging scanner (Bioscan, Washington, DC), and the production of metabolites was determined based on the chromatographic profiles. For microsomes containing the expressed CYP71A10 enzyme, control experiments were also conducted to measure the NADPH-dependency, and the inhibitory effects of CO. CO treatment of the sample was achieved by gentle bubbling of the gas through the reaction mixture for 2 minutes immediately before the assay was initiated by the addition of NADPH.

e. Enzyme Kinetics

Substrate conversion was quantified by a combination of TLC analysis and scintillation spectrometry. The location of the metabolic products on the TLC plates was identified using an imaging scanner, the bands were scraped and analyzed by scintillation spectrometry. The amount of metabolite produced was calculated based on specific activity and scintillation counts. Each assay was repeated at least twice. K_m and V_{max} values were estimated using nonlinear regression analysis.

f. Mass Spectral Analysis

The reaction components used in the *in vitro* fluometuron and linuron metabolism assays were scaled up 50-fold, and the reactions were allowed to proceed for 3 hours. The substrates and the metabolites were extracted 3 times with 20 ml ethyl acetate. The extracts were combined, evaporated to dryness, and the resulting pellet was resuspended in 1 ml acetone. The samples were purified twice using preparative TLC and imaging scanning as described above . Finally, the respective bands were scraped, the compounds were eluted with acetone and flash evaporated.

Fractions of interest were analyzed by liquid chromatography/mass

spectrometry (LC/MS). Mass spectral measurements were made with a Finnigan TSQ 7000 triple quadruple mass spectrometer (QQQ) equipped with an Atmospheric Pressure Ionization (API) interface fitted with a pneumatically assisted electrospray head (Finnigan MAT, Brennan, Germany). The spray nozzle was operated at 5 kV in the positive ion mode and 4 kV in the negative ion mode. For sample introduction, the TSQ 7000 was equipped with a HPLC solvent delivery system (Perkin-Elmer 410 LC pump), a UV detector (Perkin-Elmer), a stream splitter set at 6:1 with the majority of the effluent flowing to a radioisotope flow monitor (IN/US β-RAM) and the other stream attached to the API interface. Samples were chromatographed on a reverse phase HPLC column (Inertsil 5 ODS2, 150 x 2 mm i.d.). The column was eluted at 0.4 ml/min with 95:5 of 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in methanol, respectively. Collision induced dissociation experiments (MS/MS) were conducted using argon gas with collision energy in the range of 17.5-30 eV at cell pressures of approximately 0.28 Pa. Signals were captured using a Finnigan 7000 data system.

g. NMR Analysis

Proton NMR measurements were made on a Bruker AMX-400 NMR spectrometer equipped with either a QNP or inverse probe set at 400.13 MHZ. Spectra were acquired at ambient temperature in acetonitrile-d_3. Chemical shifts were expressed as parts per million, relative to the resonance of residual acetonitrile protons at 1.93 ppm (δ).

h. Tobacco Transformation

A plant expression vector capable of mediating the constitutive expression of CYP71A10 was produced. The GUS open reading frame of the binary expression vector pBI121 (Clontech, Polo Alto, CA) was excised and replaced with the full length CYP71A10 reading frame. This placed the soybean gene under the transcriptional control of the strong constitutive CaMV 35S promoter.

The resulting construct was used to transform Agrobacterium tumefaciens strain LBA 4404 (Holsters et al., *Mol. Gen. Genetics*, 163:181-187 (1988)). Excised leaf discs of Nicotiana tabacum cv SR1 were transformed using the Agrobacterium, and kanamycin-resistant plants were selected as described by Horsch et al. *Science*, 227:1229-1231 (1985). Primary transformants were potted in a standard soil mixture, transferred to a greenhouse and their seed harvested upon maturation.

i. *In vivo* Herbicide Metabolism Assays

Seeds from primary transgenic tobacco plants transformed with CYP71A10 and control plants transformed with the pBI121 vector were grown in Petri dishes containing MS salts and 100 µg/ml kanamycin. At five weeks post-seeding, kanamycin-resistant plantlets were transplanted into pots containing soil and grown an additional two weeks. Single leaves of approximately 10 cm^2 in size were excised and their petioles inserted into 100 µl of H_2O containing radiolabeled herbicide. The leaves were placed in a growth chamber maintaining a temperature of 27°C and incubated until the entire volume of the herbicide solution was drawn up by the transpirational stream of the leaves (about 3 hrs). The leaves were subsequently transferred into an Eppendorf tube containing distilled water and further incubated for a total of 14 hours.

[^{14}C]-labeled herbicide was extracted from the leaves by grinding for 5 minutes in 250 µl methanol with a plastic pellet pestle driven by an electric drill. After centrifugation for 3 minutes at 14,000 g, 75 µl of the supernatant was spotted on a Whatman K6F silica plate and developed in a solvent system containing chloroform/ethanol/acetic acid 135:10:15 (v/v/v). The separated herbicide derivatives were visualized using an imaging scanner. Substrate conversion was quantified based on the amount of herbicide absorbed, and the ratios of the parent compound and the produced metabolites determined from the TLC profiles.

j. Herbicide Tolerance

T_1 generation seeds from CYP71A10-transformed tobacco and pBI121-transformed control plants were placed onto Petri dishes containing MS salts and linuron (using its commercial formulation LOROX 50 DF) at active ingredient concentrations ranging from 0.25 to 3.0 μM. Chlortoluron was added at 0, 1.0, 5.0 and 10.0 μM concentrations using a 99.5% pure analytical standard. The Petri dishes were incubated in a growth chamber maintaining a constant temperature of 27°C and a 16/8 hour light/dark cycle. The phytotoxic effects of the treatments were determined visually by comparison to control plants and plants grown in the absence of the herbicide. All treatments were repeated at least twice.

EXAMPLE 2

Isolation of P-450 cDNAs

To isolate cDNAs encoding P-450s from soybean, the PCR strategy described by Meijer et al. (1993) was adapted, using a soybean leaf cDNA library as the template. Degenerate, inosine-containing PCR primers were constructed corresponding to the first nine codons encoding the conserved sequence FLPFGxGxRxCxG (x = any amino acid) (**SEQ ID NO:20**), which represents an extension of the highly conserved FxxGxxxCxG motif (Bozak et al., *Proc. Natl. Acad. Sci. USA* 87:3904-3908 (1990)) (**SEQ ID NO:21**). Located near the C-terminal end of the protein, this motif defines the heme-binding region of the protein and may be regarded as a "signature" for P-450 proteins. A second nonspecific primer complementary to the poly(A) tail of the cDNA clones was used in conjunction with these degenerate primers in a PCR amplification assay. PCR amplification products were cloned into a plasmid vector and analyzed by DNA sequencing. Of 86 randomly selected individuals that were sequenced, 15 clones representing 10 unique cDNAs were identified that possessed the conserved cysteine and glycine residues of the signature

consensus (xCxG) (SEQ ID NO:22) immediately following the sequence defined by the degenerate PCR primers. Furthermore, homology searches of the major DNA and protein data bases revealed additional sequence identities to previously reported P-450 sequences for each of the ten unique soybean sequences (data not shown). Because this strategy only allows the recovery of sequence corresponding to the C-terminal portion of the proteins, additional PCR-based techniques were utilized to obtain cDNAs possessing the entire reading frames for each clone. Full length cDNAs were isolated for eight of the 10 individual clones and a near full length cDNA was isolated for an additional clone.

The eight full length and one near full length soybean P-450 cDNAs isolated are described in **Table 1**. The nomenclature for P-450 genes is based on amino acid sequence identity. Typically, sequences sharing >40% identity are placed in the same family, >55% identity defines members of the same subfamily, and sequences that display >97% identity are assumed to represent allelic variants, although exceptions to these designations have been noted (Nelson et al., *Pharmacogenetics*, 6:1-41 (1996)). According to this system of nomenclature, all of the nine soybean cDNAs were able to be placed within existing P-450 gene families; however, three of the sequences (CYP82C1, CYP83D1 and CYP93C1) defined new subfamilies. Although an increasing number of P-450 gene products have been assigned specific enzymatic functions (reviewed in Schuler, 1996), none of the soybean cDNAs listed in **Table 1** could be placed into families for which an *in vivo* function had been determined for any of its members.

In addition to the conserved heme-binding domain described previously, all of the predicted soybean polypeptides possess slight variations of the conserved sequence PEEFxPERF (SEQ ID NO:23) located approximately 30 amino acids forward of the heme-binding motif (Hallahan et al., *Biochem. Soc. Trans.* 21:1068-1073 (1993)). Also characteristic of microsomal P-450s is the presence of an N-terminal noncleavable signal sequence that serves as the membrane anchor. Immediately following this signal-anchor segment in most

microsomal P-450s is a proline-rich region that is believed to form a hinge between the catalytic cytoplasmic domain and the hydrophobic membrane anchor (Halkier, *Phytochemistry* 43:1-21 (1996)). All of the present clones (except CYP97B2) encode proteins possessing predicted signal sequences; all individuals (except CYP97B2 and CYP82C1) contain readily identifiable proline-rich domains following the signal sequence (**Table 1**). It is the identification of both of these N-terminal motifs in the CYP83D1 encoded protein (but no Met codon) that indicates that this clone is nearly full length. Interestingly, instead of possessing a predicted signal sequence and proline-rich region, the N-terminus of the polypeptide encoded by clone CYP97B2 contains a motif characteristic of a chloroplast transit peptide (data not shown).

Table 1

Soybean P-450s Isolated Using Degenerate PCR Primers

Name	GenBank Accession #	Length (amino acids)	Closest Match	Identity* %	Membrane Anchor	Proline -rich Region
CYP71A10 (SEQ ID NO:1)	AF022157	513	CYP71A1	51.7	+	+
CYP71D10 (SEQ ID NO:3)	AF022459	510	CYP71D9	50.9	+	+
CYP77A3 (SEQ ID NO:5)	AF022464	513	CYP77A1	69.8	+	+
CYP78A3 (SEQ ID NO:7)	AF022463	523	CYP78A2	53.1	+	+
CYP82C1 (SEQ ID NO:9)	AF022461	532	CYP82A3	51.1	+	–
CYP83D1** (SEQ ID NO:11)	AF022460	516	CYP71A1**	45.7	+	+
CYP93C1 (SEQ ID NO:13)	AF022462	521	CYP93B1	44.5	+	+
CYP97B2 (SEQ ID NO:15)	AF022457	576	CYP97B1	80.8	–	–
CYP98A2 (SEQ ID NO:17)	AF022458	509	CYP98A1	69.7	+	+

*Percent identity between the predicted amino acids sequences of the given soybean P-450 cDNA and the closest match identified from a BLAST search against the major gene and protein databases.

** Although this sequence shows a best match to CYP71A1, it matches poorly to some sequences of the CYP71B subfamily. As a result, the tree cluster program places it into the CYP83 family.

EXAMPLE 3

Expression of Soybean P-450 cDNAs in Yeast

Because superfluous 5' untranslated sequences from foreign genes have been shown to be capable of impeding gene expression in yeast (Pompon, 1988), an additional PCR reaction was performed on each clone that enabled the cloning of full length P-450 open reading frames (ORFs) into the yeast expression vector pYeDP60 (V-60) without including any of the endogenous 5' nontranslated flanking sequence (see Methods). For the near full length clone CYP83D1, the 5' primer was also designed to generate an "artificial" Met start codon and a Val second codon at the 5' end of the ORF. Expression in yeast of genes cloned into the V-60 vector is mediated by the strong, galactose-inducible GAL10-CYC1 promoter (Pompon et al., 1995).

Previous studies have revealed that the heterologous expression of P-450 cDNAs in yeast can be greatly enhanced in strains that have been engineered to overexpress endogenous NADPH-dependent cytochrome P-450 reductase (Pompon et al., 1995). In strain W(R), this was accomplished by exchanging the relatively weak endogenous cytochrome P-450 reductase promoter with the same GAL10-CYC1 promoter used in vector V-60 (Truan et al., *Gene* 125:49-55 (1993)). To maximize the heterologous expression of the soybean P-450 cDNAs in yeast, each of the constructs cloned into the V-60 vector was transformed into strain W(R) and microsomes were isolated from cultures that had been induced by galactose.

Reduced-CO difference spectroscopy provides a method to measure the effectiveness of expression of heterologous P-450s in yeast. Microsomal preparations corresponding to five of the soybean constructs (CYP71A10, CYP71D10, CYP77A3, CYP83D1 and CYP98A2) showed characteristic P-450 CO difference spectra with Soret peaks at 450 nm; the profile corresponding to CYP71A10 is shown in **Figure 1**. No such peaks were observed for the remaining four clones. The specific P-450 content of the five positive

microsomal preparations varied significantly, ranging from 11 pmol P-450/mg protein for construct CYP83D1 to 252 pmol P-450/mg for clone CYP77A3 as shown in Table 2.

Table 2

P-450 Content of Microsomes Isolated from Yeast Overexpressing Various Soybean CYPs

Clone	CYP content (pmol mg^{-1} protein)
CYP71A10	44
CYP71D10	15
CYP77A3	252
CYP83D1	11
CYP98A2	13

EXAMPLE 4

In vitro Herbicide Assays

To determine whether any of the present soybean P-450 proteins synthesized in yeast displayed significant herbicide metabolic activity, microsomal preparations possessing each of the five soybean P-450s that were effectively expressed in yeast (as judged by their reduced CO difference spectra, see above) were incubated individually with NADPH and radioisotopes of the compounds listed in Table 3. These substrates represent six different classes of herbicides and one organophosphate insecticide (diazinon). Upon termination of the reaction, each sample was analyzed by thin layer chromatography (TLC) to reveal potential metabolic breakdown products.

The P-450 proteins expressed from clones CYP71D10, CYP77A3, CYP83D1, and CYP98A2 displayed no apparent *in vitro* metabolic activity against any of the 11 compounds tested (data not shown). In contrast, the P-450 enzyme produced from construct CYP71A10 demonstrated considerable activity

against the phenylurea class of herbicides, but no activity against the remaining compounds. As shown in **Figure 2**, fluometuron and diuron were converted to a single metabolite; linuron and chlortoluron were transformed into two (a major and a minor) metabolites. **Figure 3** shows the chemical structures of the four phenylurea herbicides tested in this study, and the derivatives that have previously been characterized as the first metabolites produced during the detoxification of the respective herbicides in plants known to metabolize these compounds (Voss and Geissbühler, *Proc. Brit. Weed Contr. Conf.* 8:266-268 (1966); Suzuki and Casida, *J. Agric. Food Chem.* 29:1027 (1981); Ryan et al., *Pestic. Biochem. Physiol.* 16:213-221 (1981)).

To further confirm that the herbicide metabolism measured from microsomes of yeast expressing CYP71A10 was attributable to a P-450 activity, additional assays utilizing linuron as the substrate were conducted. As shown in **Figure 4**, linuron metabolizing activity is reduced approximately 37% in the presence of CO, and no metabolites are observed when NADPH is omitted from the reaction. Activity is also completely abolished upon addition of tetcyclasis, a potent P-450 inhibitor (data not shown). Furthermore, no activity is detected when microsomal preparations are used from yeast cells expressing only the V-60 control plasmid. These results verify that the observed herbicide metabolizing activity is derived from the soybean CYP71A10 cDNA.

The kinetic properties and catalytic activities of the soybean CYP71A10 protein enzyme differed significantly among the four phenylurea-type herbicide substrates. As shown in **Table 4**, turnover rates for fluometuron and linuron were considerably greater than those observed for chlortoluron and diuron. The observed reduced activity for the later two substrates is apparently not the result of decreased binding affinities since the apparent K_ms for chlortoluron and diuron are lower than those measured for fluometuron and linuron.

Table 3

Compounds Used in Metabolism Assays

Common Name	Chemical Family
Alachlor	Acetanilide
Metolachlor	Acetanilide
Bentazon	Benzothiadiazole
Imazaquin	Imidazolinone
Chlortoluron	Phenylurea
Diuron	Phenylurea
Fluometuron	Phenylurea
Linuron	Phenylurea
Prosulfuron	Sulfonylurea
Metribuzin	*as*-Triazine
Diazinon	Organophosphate

Table 4

In Vitro Kinetic Parameters of the CYP71A10 Enzyme for Four Phenylurea Substrates

Substrate	$K_{m, app}$ (μM)	V_{max} (pmol min^{-1} mg^{-1} protein)	Turnover (min^{-1})
Fluometuron	14.9 (1.0)*	303.6 (10.8)	6.8 (0.24)
Linuron	9.8 (2.1)	125.6 (12.0)	2.8 (0.27)
Chlortoluron	1.0 (0.2)	29.4 (2.2)	0.7 (0.05)
Diuron	1.5 (0.3)	16.8 (1.6)	0.4 (0.04)

* Values in parentheses represent standard error.
- Assays were repeated three times for linuron and twice for all other substrates.
- Concentration ranges (μM) used were 3.2-27.7 for fluometuron, 3.8-28.3 for linuron, 0.7-4.0 for chlortoluron, and 0.7-3.7 for diuron.

EXAMPLE 5

Analysis of Metabolites

As shown in **Figure 2**, CYP71A10-mediated degradation of phenylurea herbicides resulted in the accumulation of either one or two metabolites, depending on the particular substrate used. To determine the structure of the metabolites, the single metabolite observed in the fluometuron assay and both the major and minor metabolites generated in the linuron assay were analyzed by liquid chromatography/mass spectroscopy (LC/MS) analysis (results not shown). Analysis of the fluometuron metabolite by LC/MS in positive ion mode resulted in pseudomolecular ions at m/z 219 [$(M+H)^+$, $C_9H_9F_3N_2O$] and m/z 241 $(M+Na)^+$ that corresponds to a sodium adduct. Daughter ion spectra of m/z 219 produced a prominent m/z 162 fragment ion due to formation of the protonated trifluoromethylaniline $(C_7H_6F_3N+H)^+$. Analysis of the fluometuron metabolite by proton NMR showed a singlet at δ2.71 which integrated for 3 protons (data not shown). The NMR spectra aromatic resonances were similar to aromatic resonances observed in the parent molecule. Spectra of the fluometuron metabolite were consistent for loss of a methyl group from the parent compound.

The major linuron metabolite analyzed by LC/MS in the negative ion mode showed a pseudomolecular ion at m/z 233 $(M-H)^-$ and m/z 235 $[(M+2)-H]^-$ consistent for a molecule containing two chlorine atoms. Daughter ion spectrum at m/z 233 showed a prominent fragment ion at m/z 160 $(C_6H_4Cl_2N-H)^-$. The major linuron metabolite was 15 mass units less than parent compound which is consistent with loss of a methyl group. The position of methyl loss could not be determined based on mass spectral data alone.

The minor linuron metabolite analyzed by LC/MS gave a pseudomolecular ion at m/z 217 $(M-H)^-$ and m/z 219 $[(M+2)-H]^-$ which is consistent for a molecule containing two chlorine atoms. The daughter ion spectrum at m/z 217 showed a prominent fragment ion at m/z 160 which corresponds to formation of the dichloroaniline. The mass spectral data is consistent for the minor linuron metabolite representing N-demethoxy linuron.

These results suggest that the CYP71A10 enzyme expressed in yeast produces the same fluometuron and linuron metabolites as depicted in **Figure 3**, which shows the first metabolites produced during the detoxification of the respective herbicides in plants that are known to degrade these compounds. The metabolites of chlortoluron and diuron have not been analyzed directly, but the R_f values of the peaks observed during TLC separation are consistent with these species also representing the compounds shown in **Figure 3** (ring-hydroxymethyl chlortoluron, N-demethyl chlortoluron and N-demethyl diuron). These results indicate that the CYP71A10 enzyme functions primarily as an N-demethylase with respect to fluometuron, linuron and diuron, with some N-demethoxylase activity also observed with linuron. Using chlortoluron as a substrate, the enzyme apparently functions primarily as a methyl-ring hydroxylase and to a lesser extent as an N-demethylase.

EXAMPLE 6

Herbicide Metabolism in Transgenic Tobacco

To determine whether overexpression of the soybean CYP71A10 cDNA

in a higher plant system enhances metabolism of phenylurea herbicides, the GUS gene in the binary vector pBI121 was excised and replaced with the CYP71A10 reading frame. This construct placed the CYP71A10 cDNA under the transcriptional control of the constitutive 35S promoter of Cauliflower Mosaic Virus; kanamycin selection was facilitated via the nptII selectable marker. Agrobacterium-mediated transformation of Nicotiana tabacum cv SR1 leaf discs resulted in the recovery of several dozen independent kanamycin-resistant transformants. The plants were subsequently grown to maturity in a greenhouse and allowed to set seed.

For the herbicide metabolism assays, seeds from one randomly selected transgenic line, designated 25/2, were germinated on kanamycin-containing media to eliminate potential nontransgenic segregants. Of 17 germinated seedlings grown, only one individual was inhibited by kanamycin (data not shown). This result suggests that line 25/2 possesses more than one independently segregating transgene. Individual leaves from the 25/2 progeny were excised and incubated with radiolabeled phenylurea herbicides. As shown in **Table 5**, leaves of the kanamycin-resistant individuals of line 25/2 metabolized all of the four herbicides tested to a much greater extent than the pBI121-transformed control plants.

The relative migrations of the metabolic products revealed by TLC suggest that the products observed in the *in vivo* excised leaf assay are primarily the same as were generated from the *in vitro* assays using yeast microsomes for fluometuron, linuron and diuron (data not shown). For chlortoluron, additional metabolites were also observed. These likely represent combinations of ring-methyl hydroxylated and mono- and di-demethylated species as had been observed by Shiota et al. *Pestic. Biochem. Physiol.* 54:190-198 (1996), in their analysis of chlortoluron-resistant transgenic tobacco that overexpressed the rat CYP1A1 gene. Differences in the ratios of the observed chlortoluron metabolites were also observed between the CYP71A10-transformed and the control plants. Sixty three percent of the metabolites produced in the control leaves was N-

demethyl chlortoluron; in contrast, ring-methyl hydroxy chlortoluron was the most abundant metabolite generated in the CYP71A10-transformed leaves (47%) and only 8% of the metabolites represented N-demethyl chlortoluron.

Table 5

Phenylurea Metabolism after 14 Hours by Excised Leaves of Transgenic Tobacco Plant 25/2 Progeny

Herbicide[a]	CYP71A10-transformed	Control[b]
	% of herbicide metabolized	
Fluometuron	91 (4.5)[c]	15 (0.6)
Linuron	87 (2.0)	12 (2.6)
Chlortoluron	85 (8.1)[d]	39 (7.5)[d]
Diuron	49 (7.0)	20 (2.0)

(a) Equal amounts of herbicide (1.2 nmol) were added for each experiment.

(b) Plants transformed with the pBI121 construct were used as controls.

(c) Values in parentheses represent standard error. A single leaf was assayed from four independent 25/2 plants and three independent control plants.

(d) The major chlortoluron metabolite in the control plants represented N-demethyl chlortoluron (63%). The metabolites recovered from the CYP71A10-transformed leaves were ring-methyl hydroxy chlortoluron (47%), N-demethyl chlortoluron (8%) and other derivatives (45%).

EXAMPLE 7

Herbicide Tolerance

To establish whether enhanced herbicide metabolism leads to an increase in tolerance at the whole plant level, seeds from transgenic plant 25/2 were germinated on an agarose-base medium containing MS salts and varying

concentrations of linuron. Growth of wild-type SR1 plants and transgenic control plants expressing the GUS gene (from vector pBI121) was severely inhibited when exposed to 0.25 μM linuron and completely arrested at concentrations of 0.5 μM and higher (data not shown). As shown in **Figure 5**, progeny of plant 25/2 grown on media containing no herbicide (**Figure 5A**) appeared indistinguishable from the same seed grown in the presence of 0.5 μM linuron (**Figure 5C**), where only one of 23 germinated seedlings appeared to be inhibited by the herbicide. This ratio appears to be consistent with that observed when seeds from the same parent were grown on selective media containing kanamycin; only one of 17 seedlings failed to grow in the presence of kanamycin. **Figure 5B** shows control tobacco plants (transformed with vector pBI121), grown on media containing 0.5μM linuron. 25/2 plants tolerant to linuron levels as high as 2.5 μM linuron were observed, although an increasing percentage of the plants showed growth inhibition as the herbicide concentration was increased (**Figure 5D**). Segregation of the transgene(s) may be leading to variability in expression levels among the progeny of 25/2.

To examine whether the acquisition of herbicide tolerance is unique to line 25/2, seeds from 20 other independent CYP71A10-expressing transgenic plants were similarly germinated and grown on media containing 0.5 μM linuron. Of these, 19 lines gave rise to progeny that were linuron tolerant. The percentage of tolerant individuals for each line varied from approximately 20% to 100% (data not shown). This variation likely represents differences in the copy number, expression levels and segregation of the transgene among the independent lines.

Chlortoluron-tolerance of line 25/2 was also evident. At 1.0 μM herbicide concentration chlortoluron completely arrested the growth of the control plants (**Figure 5E**). Although growth of the 25/2 plants was modestly inhibited at this herbicide concentration, with the exception of two presumably nontransgenic segregants, the CYP71A10-transformed plants appeared healthy (**Figure 5F**). In contrast to linuron and chlortoluron, little tolerance of line 25/2

to fluometuron or diuron was observed. Herbicide concentrations that were injurious to the control plants also inhibited the growth of line 25/2 individuals. Enhanced fluometuron or diuron tolerance was only observed at the very lowest herbicide concentrations necessary to impose growth inhibition in the control plants (data not shown).

THAT WHICH IS CLAIMED IS:

1. An isolated DNA molecule comprising a sequence selected from the group consisting of:

a) SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, and SEQ ID NO:17;

b) DNA sequences which encode an enzyme having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO6:, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, and SEQ ID NO:18;

c) DNA sequences which have at least about 90% sequence identity to the DNA of (a) or (b) above and which encode a cytochrome P450 enzyme; and

d) DNA sequences which differ from the DNA of (a) or (c) above due to the degeneracy of the genetic code.

2. A peptide encoded by a DNA sequence of claim 1.

3. A cytochrome p450 enzyme having an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO6:, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, and SEQ ID NO:18.

4. An isolated DNA molecule comprising a sequence selected from the group consisting of:

a) SEQ ID NO:1;

b) DNA sequences which encode an enzyme having SEQ ID NO:2,;

c) DNA sequences which have at least about 90% sequence identity to the DNA of (a) or (b) above and which encode a cytochrome P450 enzyme; and

d) DNA sequences which differ from the DNA of (a) or (c) above due to the degeneracy of the genetic code.

5. A peptide encoded by a DNA sequence of claim 4.

6. A cytochrome p450 peptide having SEQ ID NO:2.

7. A DNA construct comprising an expression cassette, which construct comprising in the 5' to 3' direction, a promoter operable in a plant cell and a DNA segment according to claim 1 positioned downstream from said promoter and operatively associated therewith.

8. A DNA construct according to claim 7, wherein said promoter is constitutively active in plant cells.

9. A DNA construct according to claim 7, wherein said promoter is the 35S promoter from Cauliflower Mosaic virus.

10. A DNA construct according to claim 7, said construct further comprising a plasmid.

11. A DNA construct according to claim 7 carried by a plant transformation vector.

12. A DNA construct according to claim 7 carried by an *Agrobacterium tumefaciens* plant transformation vector.

13. A plant cell containing a DNA construct according to claim 7.

14. A transgenic plant comprising plant cells according to claim 13.

15. A transgenic plant according to claim 14, wherein said plant is a monocot.

16. A transgenic plant according to claim 14, wherein said plant is a dicot.

17. A DNA construct comprising an expression cassette, which construct comprising in the 5' to 3' direction, a promoter operable in a plant cell, and a DNA segment encoding a peptide of SEQ ID NO:2 positioned downstream from said promoter and operatively associated therewith.

18. A DNA construct according to claim 17, wherein said promoter is constitutively active in plant cells.

19. A DNA construct according to claim 17, wherein said promoter is the 35S promoter from Cauliflower Mosaic virus.

20. A DNA construct according to claim 17, said construct further comprising a plasmid.

21. A DNA construct according to claim 17 carried by a plant transformation vector.

22. A DNA construct according to claim 17 carried by an *Agrobacterium tumefaciens* plant transformation vector.

23. A plant cell containing a DNA construct according to claim 17.

24. A transgenic plant comprising plant cells according to claim 23.

25. A transgenic plant according to claim 24, wherein said plant is a monocot.

26. A transgenic plant according to claim 24, wherein said plant is a dicot.

27. A method of making a transgenic plant cell having an increased ability to metabolize phenylurea compounds compared to an untransformed plant cell, said method comprising:

a) providing a plant cell;

b) transforming said plant cell with an exogenous DNA construct comprising, in the 5' to 3' direction, a promoter operable in a plant cell and a DNA sequence encoding a peptide of SEQ ID NO:2, said DNA sequence operably linked to said promoter.

28. A method according to claim 27, wherein said plant cell is from a member of the Solanacae family.

29. A method according to claim 27, wherein said promoter is the 35S promoter from Cauliflower Mosaic virus.

30. A method according to claim 27, wherein said transforming step is carried out by bombarding said plant cell with microparticles carrying said DNA construct.

31. A method according to claim 27 wherein said transforming step is carried out by infecting said plant cell with an *Agrobacterium tumefaciens* containing a Ti plasmid carrying said DNA construct.

32. A method according to claim 27, further comprising regenerating a plant from said transformed plant cell.

33. A transformed plant produced by the method of claim 32.

34. Seed or progeny of a plant according to claim 33, which seed or progeny has inherited said DNA sequence encoding a peptide of SEQ ID NO:2.

35. A transformed plant produced by the method of claim 32, which plant has increased resistance to phenylurea herbicides compared to wild-type plants of the same species.

36. A transgenic plant having an increased ability to metabolize phenylurea compounds compared to an untransformed plant cell, said transgenic plant comprising transgenic plant cells containing an exogenous DNA construct comprising, in the 5' to 3' direction, a promoter operable in said plant cell, said promoter operably linked to a DNA sequence encoding a peptide of SEQ ID NO:2.

37. A transgenic plant according to claim 36, wherein said promoter is the 35S promoter from Cauliflower Mosaic virus.

38. A transgenic plant according to claim 36, wherein said plant is a dicot.

39. A transgenic plant according to claim 36, wherein said plant is a monocot.

40. A transgenic plant according to claim 36, wherein said plant is a member of the family Solanacae.

41. A transgenic plant according to claim 36, which plant is selected from the group consisting of tobacco, potato, tomato, corn, rice, cotton, soybean,

rape, wheat, oats, barley, rye and rice.

42. Progeny or seed of a plant according to claim 36, wherein said seed or progeny has inherited said DNA sequence encoding a peptide of SEQ ID NO:2.

43. A transformed plant according to claim 36, which plant has increased resistance to phenylurea herbicides compared to wild-type plants of the same species.

44. A crop comprising a plurality of plants according to claim 36 planted in an agricultural field.

45. A method of using a phenylurea herbicide as a post-emergence herbicide, comprising:

a) planting a crop according to claim 44;

b) applying to said crop a phenylurea herbicide.

46. A method according to claim 45, wherein said crop is selected from the group consisting of turfgrass, tobacco, potato, tomato, corn, rice, cotton, soybean, rape, wheat, oats, barley, rye and rice.

47. A method according to claim 45, wherein said herbicide is selected from the group consisting of fluometuron, linuron, chlortoluron and diuron.

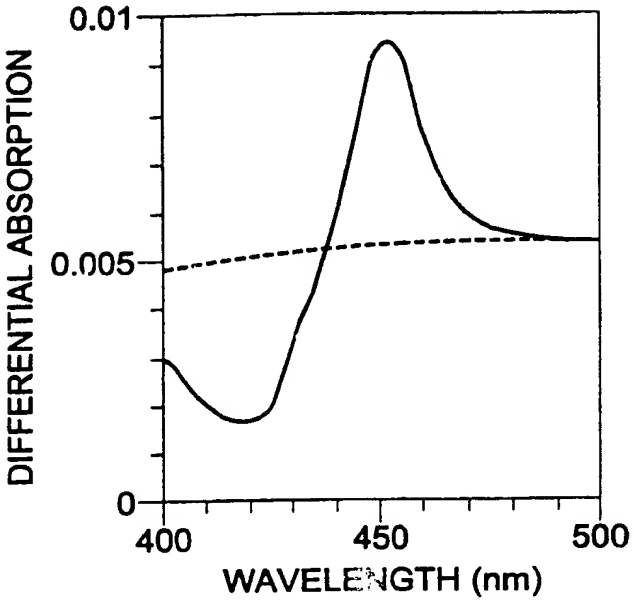


FIG. 1.

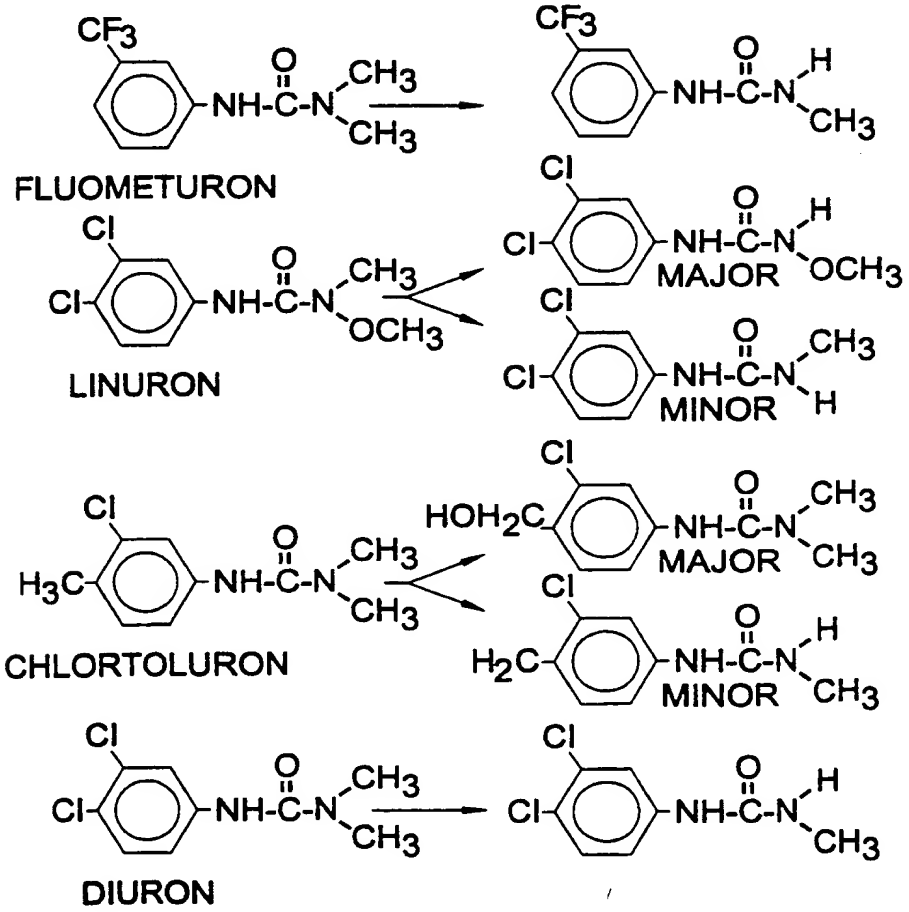


FIG. 3.

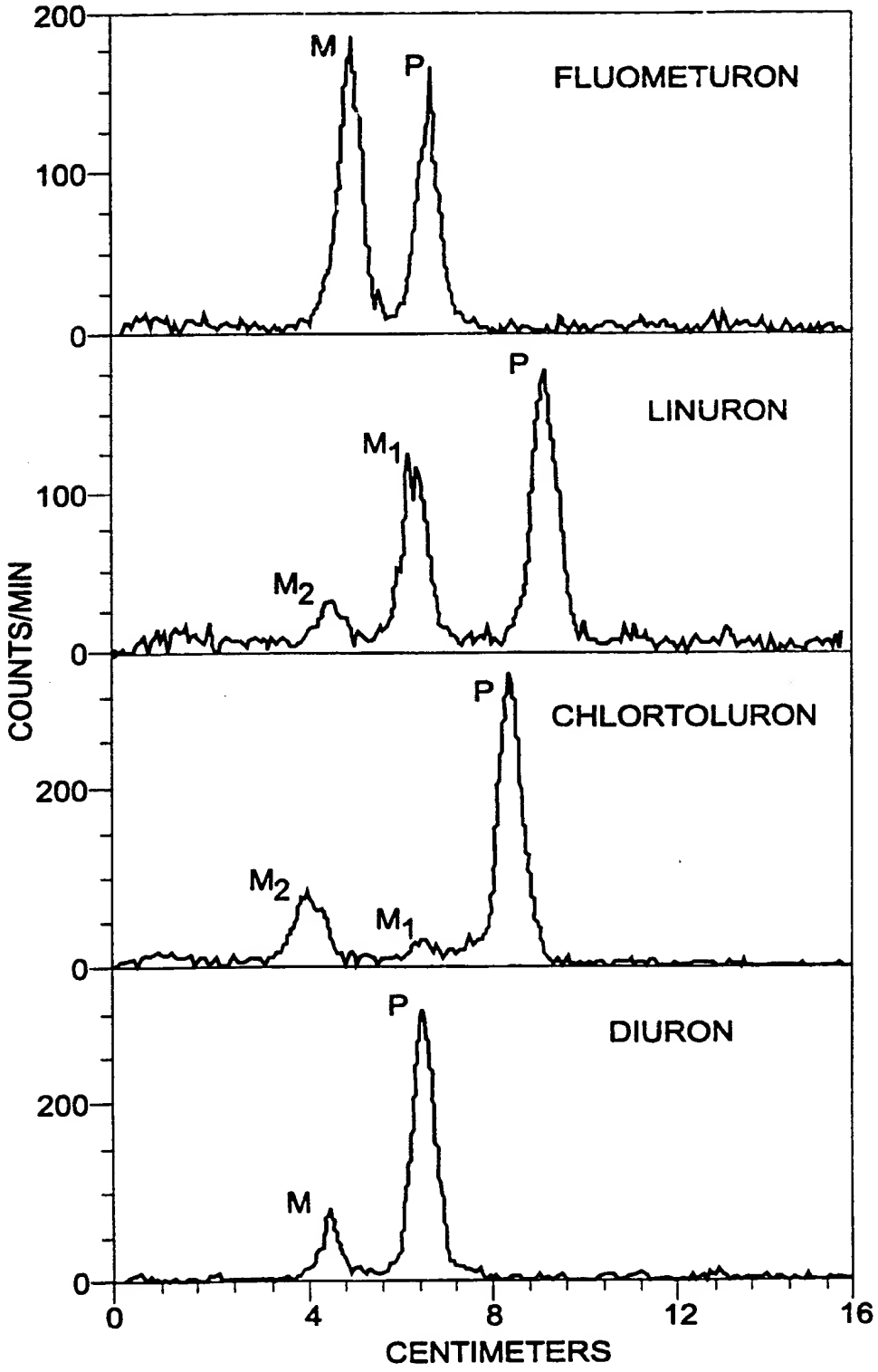


FIG. 2.

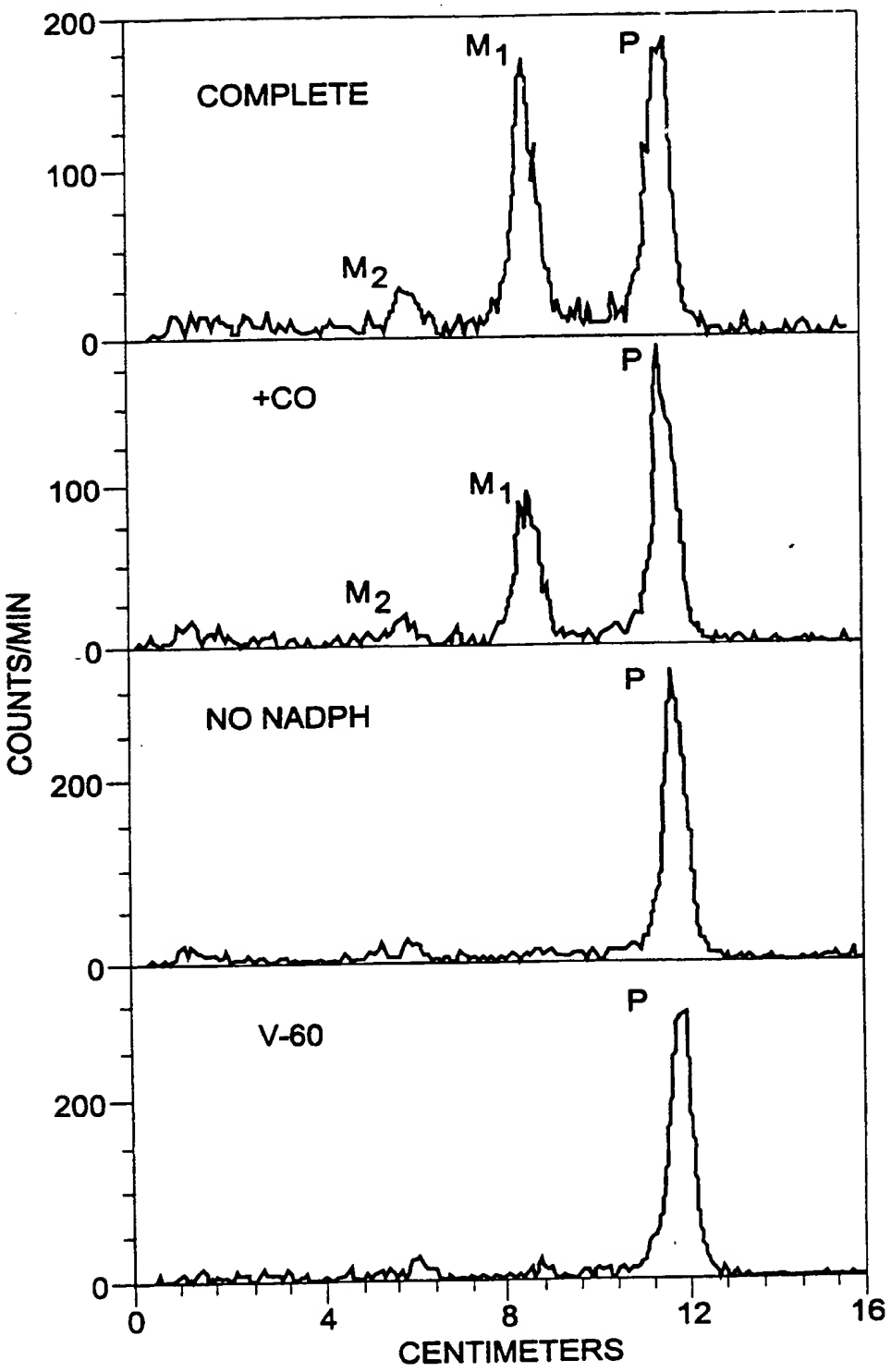


FIG. 4.

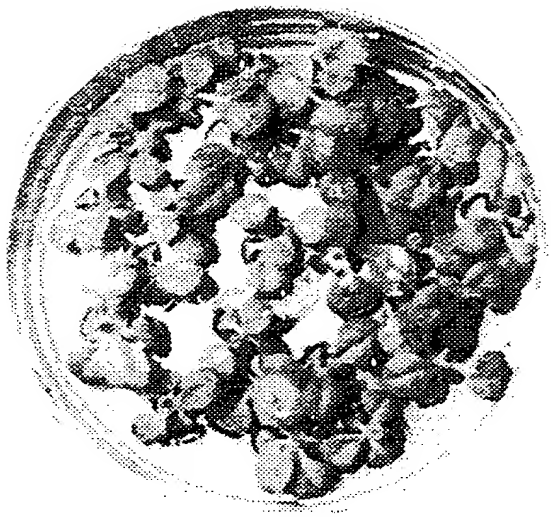


FIG. 5A.

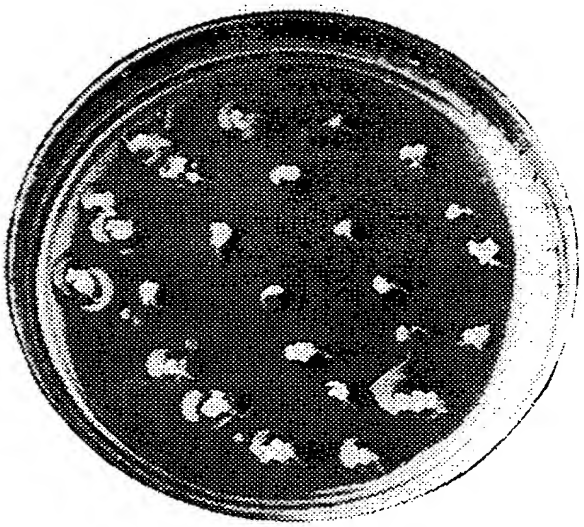


FIG. 5B.

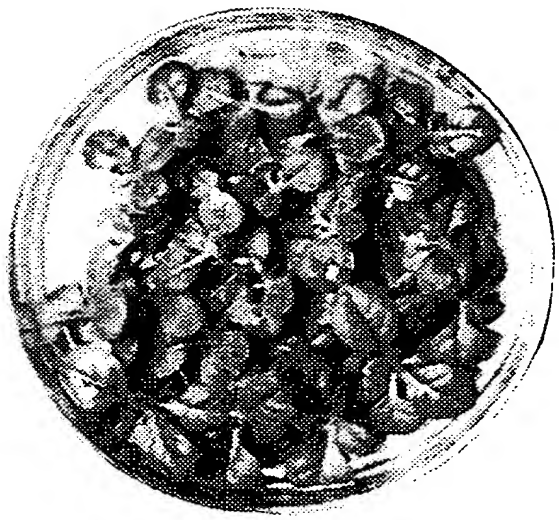
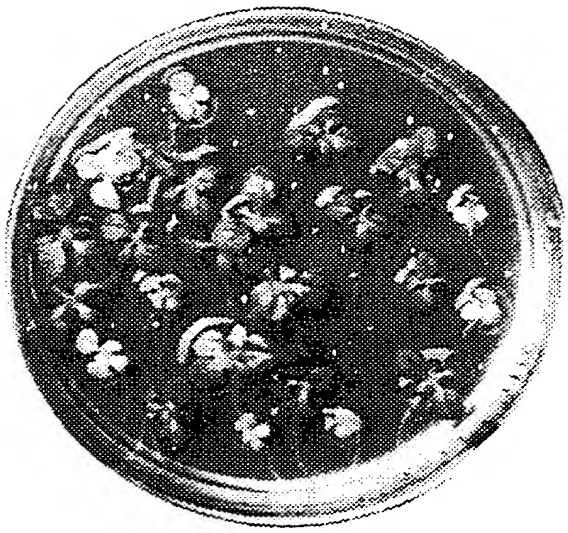


FIG. 5C.

FIG. 5D.



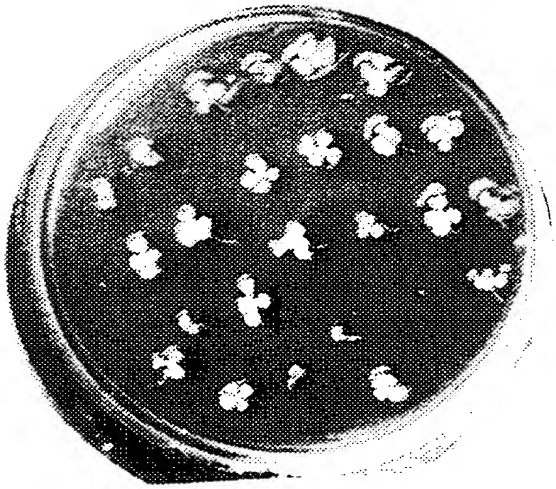


FIG. 5E.

FIG. 5F.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Siminszky, Balazs
Dewey, Ralph E.
Corbin, Frederick T.

(ii) TITLE OF INVENTION: Novel Cytochrome P-450 Constructs and Methods of Producing Herbicide-Resistant Transgenic Plants

(iii) NUMBER OF SEQUENCES: 23

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Virginia C. Bennett
(B) STREET: PO Box 37428
(C) CITY: Raleigh
(D) STATE: North Carolina
(E) COUNTRY: USA
(F) ZIP: 27627

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Bennett, Virginia C.
(B) REGISTRATION NUMBER: 37,092
(C) REFERENCE/DOCKET NUMBER: 5051-409

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 919-854-1400
(B) TELEFAX: 919-854-1401

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1838 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 4..1542

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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      1               5                  10                  15

AGT TCA ACC CAT TAC CTA ACA GTT TTC TTC TGC ATC TTC CTT ATA CTT          96
Ser Ser Thr His Tyr Leu Thr Val Phe Phe Cys Ile Phe Leu Ile Leu
                  20                  25                  30

CTT CAG CTA ATA AGA AGA AAC AAA TAC AAT CTG CCA CCA TCC CCA CCA         144
Leu Gln Leu Ile Arg Arg Asn Lys Tyr Asn Leu Pro Pro Ser Pro Pro
              35                  40                  45

AAG ATA CCC ATA ATC GGC AAT CTT CAC CAG CTA GGC ACA CTG CCA CAC         192
Lys Ile Pro Ile Ile Gly Asn Leu His Gln Leu Gly Thr Leu Pro His
          50                  55                  60

CGC TCC TTT CAT GCA CTC TCA CAC AAA TAT GGC CCT CTC ATG ATG TTG         240
Arg Ser Phe His Ala Leu Ser His Lys Tyr Gly Pro Leu Met Met Leu
      65                  70                  75

CAA TTG GGT CAA ATT CCA ACC CTA GTG GTC TCA TCA GCT GAC GTG GCC         288
Gln Leu Gly Gln Ile Pro Thr Leu Val Val Ser Ser Ala Asp Val Ala
  80                  85                  90                  95

AGA GAA ATA ATC AAA ACG CAT GAT GTT GTT TTC TCC AAC CGC CGA CAA         336
Arg Glu Ile Ile Lys Thr His Asp Val Val Phe Ser Asn Arg Arg Gln
                 100                 105                 110

CCT ACA GCT GCT AAA ATC TTT GGT TAT GGA TGC AAA GAT GTG GCT TTC         384
Pro Thr Ala Ala Lys Ile Phe Gly Tyr Gly Cys Lys Asp Val Ala Phe
             115                 120                 125

GTG TAC TAC CGC GAA GAG TGG AGA CAA AAG ATA AAG ACA TGT AAG GTT         432
Val Tyr Tyr Arg Glu Glu Trp Arg Gln Lys Ile Lys Thr Cys Lys Val
         130                 135                 140

GAG CTT ATG AGT CTG AAG AAG GTG CGG TTG TTT CAT TCC ATT AGA CAA         480
Glu Leu Met Ser Leu Lys Lys Val Arg Leu Phe His Ser Ile Arg Gln
     145                 150                 155

GAA GTT GTT ACA GAG TTG GTT GAA GCT ATA GGT GAA GCG TGT GGT AGT         528
Glu Val Val Thr Glu Leu Val Glu Ala Ile Gly Glu Ala Cys Gly Ser
160                 165                 170                 175

GAA AGA CCA TGT GTG AAT CTG ACT GAG ATG CTG ATG GCA GCA TCG AAC         576
Glu Arg Pro Cys Val Asn Leu Thr Glu Met Leu Met Ala Ala Ser Asn
                 180                 185                 190

GAC ATT GTG TCT AGA TGT GTT CTT GGA CGG AAG TGT GAT GAT GCA TGT         624
Asp Ile Val Ser Arg Cys Val Leu Gly Arg Lys Cys Asp Asp Ala Cys
             195                 200                 205

GGT GGT AGT GGC AGT AGC AGC TTT GCA GCG TTG GGA AGA AAG ATT ATG         672
Gly Gly Ser Gly Ser Ser Ser Phe Ala Ala Leu Gly Arg Lys Ile Met
         210                 215                 220

AGA CTA TTA TCG GCT TTC AGC GTG GGT GAT TTC TTC CCT TCG TTG GGT         720
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Arg Leu Leu Ser Ala Phe Ser Val Gly Asp Phe Phe Pro Ser Leu Gly
225                 230                 235

TGG GTT GAC TAT CTG ACT GGC TTA ATT CCA GAG ATG AAA ACC ACG TTT      768
Trp Val Asp Tyr Leu Thr Gly Leu Ile Pro Glu Met Lys Thr Thr Phe
240                 245                 250                 255

CTC GCA GTA GAT GCT TTC CTT GAT GAG GTA ATT GCA GAA CAC GAG AGC      816
Leu Ala Val Asp Ala Phe Leu Asp Glu Val Ile Ala Glu His Glu Ser
                260                 265                 270

AGT AAC AAG AAG AAT GAT GAC TTC TTG GGG ATA CTT CTT CAA CTT CAA      864
Ser Asn Lys Lys Asn Asp Asp Phe Leu Gly Ile Leu Leu Gln Leu Gln
            275                 280                 285

GAA TGT GGG AGG CTT GAC TTT CAG CTC GAC CGA GAT AAC CTC AAA GCA      912
Glu Cys Gly Arg Leu Asp Phe Gln Leu Asp Arg Asp Asn Leu Lys Ala
        290                 295                 300

ATC CTA GTG GAC ATG ATA ATA GGT GGG AGT GAC ACT ACT TCA ACA ACT      960
Ile Leu Val Asp Met Ile Ile Gly Gly Ser Asp Thr Thr Ser Thr Thr
305                 310                 315

CTA GAA TGG ACT TTT GCG GAG TTC CTT AGA AAT CCA AAT ACC ATG AAG     1008
Leu Glu Trp Thr Phe Ala Glu Phe Leu Arg Asn Pro Asn Thr Met Lys
320                 325                 330                 335

AAA GCT CAA GAA GAG GTA AGA AGA GTG GTG GGA ATC AAT TCC AAA GCA     1056
Lys Ala Gln Glu Glu Val Arg Arg Val Val Gly Ile Asn Ser Lys Ala
                340                 345                 350

GTA CTG GAT GAA AAT TGT GTG AAT CAA ATG AAC TAC TTG AAA TGT GTA     1104
Val Leu Asp Glu Asn Cys Val Asn Gln Met Asn Tyr Leu Lys Cys Val
            355                 360                 365

GTC AAA GAA ACT TTG AGA TTA CAT CCA CCC CTT CCT CTT TTG ATT GCT     1152
Val Lys Glu Thr Leu Arg Leu His Pro Pro Leu Pro Leu Leu Ile Ala
        370                 375                 380

CGA GAG ACA TCA TCA AGT GTA AAA CTA AGA GGG TAC GAT ATT CCC GCA     1200
Arg Glu Thr Ser Ser Ser Val Lys Leu Arg Gly Tyr Asp Ile Pro Ala
385                 390                 395

AAA ACA ATG GTA TTT ATC AAT GCA TGG GCG ATC CAG AGG GAT CCT GAA     1248
Lys Thr Met Val Phe Ile Asn Ala Trp Ala Ile Gln Arg Asp Pro Glu
400                 405                 410                 415

TTA TGG GAT GAT CCT GAA GAA TTT ATT CCC GAA AGA TTT GAA ACT AGC     1296
Leu Trp Asp Asp Pro Glu Glu Phe Ile Pro Glu Arg Phe Glu Thr Ser
                420                 425                 430

CAA GTT GAT CTT AAT GGA CAA GAT TTT CAA TTA ATT CCG TTC GGT ATT     1344
Gln Val Asp Leu Asn Gly Gln Asp Phe Gln Leu Ile Pro Phe Gly Ile
            435                 440                 445

GGG AGA AGG GGA TGC CCT GCA ATG TCA TTT GGA CTT GCT TCA ACT GAG     1392
Gly Arg Arg Gly Cys Pro Ala Met Ser Phe Gly Leu Ala Ser Thr Glu
        450                 455                 460
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TAT GTT CTT GCT AAT CTT TTG TAT TGG TTC AAT TGG AAT ATG TCC GAG      1440
Tyr Val Leu Ala Asn Leu Leu Tyr Trp Phe Asn Trp Asn Met Ser Glu
    465                 470                 475

TCT GGA CGT ATA TTG ATG CAC AAC ATT GAC ATG AGT GAG ACA AAT GGA      1488
Ser Gly Arg Ile Leu Met His Asn Ile Asp Met Ser Glu Thr Asn Gly
480                 485                 490                 495

CTC ACT GTC AGT AAG AAA GTA CCA CTT CAT CTT GAA CCA GAA CCA TAT      1536
Leu Thr Val Ser Lys Lys Val Pro Leu His Leu Glu Pro Glu Pro Tyr
                500                 505                 510

AAA ACA TGATCATTTC ACATTATGCA TGTTTGGCAA CACCTATAAA GAGTATAGAT       1592
Lys Thr

CTGGAAGTAC TTCAATTTAG TAATGGATGT AAAAGCTATA CAATAAGAAG TGCTAACAAG    1652

CTAGGATATG AGCATTTATG GAGTAACGAG TGAGGTTCCA AAGAGTCTAA TTACTCGTCT    1712

CTTGAACATT GTTATATTTG TTTTCTTGCA GTTTGTTAAT CTTTTGAATA GTTGTTTCAC    1772

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GTTCTT                                                               1838
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 513 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Ala Leu Leu Ser Ser Val Leu Lys Gln Leu Pro His Glu Leu Ser
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            20                  25                  30

Gln Leu Ile Arg Arg Asn Lys Tyr Asn Leu Pro Pro Ser Pro Pro Lys
        35                  40                  45

Ile Pro Ile Ile Gly Asn Leu His Gln Leu Gly Thr Leu Pro His Arg
    50                  55                  60

Ser Phe His Ala Leu Ser His Lys Tyr Gly Pro Leu Met Met Leu Gln
65                  70                  75                  80

Leu Gly Gln Ile Pro Thr Leu Val Val Ser Ser Ala Asp Val Ala Arg
                85                  90                  95

Glu Ile Ile Lys Thr His Asp Val Val Phe Ser Asn Arg Arg Gln Pro
            100                 105                 110

Thr Ala Ala Lys Ile Phe Gly Tyr Gly Cys Lys Asp Val Ala Phe Val
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115 120 125

Tyr Tyr Arg Glu Glu Trp Arg Gln Lys Ile Lys Thr Cys Lys Val Glu
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Leu Met Ser Leu Lys Lys Val Arg Leu Phe His Ser Ile Arg Gln Glu
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Arg Pro Cys Val Asn Leu Thr Glu Met Leu Met Ala Ala Ser Asn Asp
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Ile Val Ser Arg Cys Val Leu Gly Arg Lys Cys Asp Asp Ala Cys Gly
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Gly Ser Gly Ser Ser Ser Phe Ala Ala Leu Gly Arg Lys Ile Met Arg
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325 330 335

Ala Gln Glu Glu Val Arg Arg Val Val Gly Ile Asn Ser Lys Ala Val
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405 410 415

Trp Asp Asp Pro Glu Glu Phe Ile Pro Glu Arg Phe Glu Thr Ser Gln
420 425 430

Val Asp Leu Asn Gly Gln Asp Phe Gln Leu Ile Pro Phe Gly Ile Gly

435 440 445

Arg Arg Gly Cys Pro Ala Met Ser Phe Gly Leu Ala Ser Thr Glu Tyr
450 455 460

Val Leu Ala Asn Leu Leu Tyr Trp Phe Asn Trp Asn Met Ser Glu Ser
465 470 475 480

Gly Arg Ile Leu Met His Asn Ile Asp Met Ser Glu Thr Asn Gly Leu
485 490 495

Thr Val Ser Lys Lys Val Pro Leu His Leu Glu Pro Glu Pro Tyr Lys
500 505 510

Thr

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1691 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 16..1545

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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15 20 25

TTA GTT CAA AGA TCG GAT TCC AAA ACC TCC TCT ACC TGC AAA TTG CCC 147
Leu Val Gln Arg Ser Asp Ser Lys Thr Ser Ser Thr Cys Lys Leu Pro
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CCA GGA CCA AGG ACA CTA CCT CTC ATA GGG AAC ATA CAC CAG ATT GTT 195
Pro Gly Pro Arg Thr Leu Pro Leu Ile Gly Asn Ile His Gln Ile Val
45 50 55 60

GGC TCA CTG CCG GTT CAT TAC TAC TTA AAA AAT TTG GCA GAT AAG TAT 243
Gly Ser Leu Pro Val His Tyr Tyr Leu Lys Asn Leu Ala Asp Lys Tyr
65 70 75

GGT CCA TTA ATG CAT CTA AAA CTA GGA GAG GTG TCC AAC ATC ATA GTC 291
Gly Pro Leu Met His Leu Lys Leu Gly Glu Val Ser Asn Ile Ile Val
80 85 90

ACT TCC CCA GAA ATG GCC CAA GAG ATT ATG AAG ACA CAT GAT CTC AAC 339

```
Thr Ser Pro Glu Met Ala Gln Glu Ile Met Lys Thr His Asp Leu Asn
          95                  100                 105

TTC TCT GAT AGG CCA GAC TTT GTA TTG TCT AGA ATA GTT TCT TAC AAC      387
Phe Ser Asp Arg Pro Asp Phe Val Leu Ser Arg Ile Val Ser Tyr Asn
    110                 115                 120

GGT TCT GGC ATT GTC TTC AGT CAA CAT GGA GAC TAT TGG AGG CAA CTA      435
Gly Ser Gly Ile Val Phe Ser Gln His Gly Asp Tyr Trp Arg Gln Leu
125                 130                 135                 140

AGA AAG ATA TGC ACA GTA GAG TTA CTA ACA GCA AAG CGC GTG CAG TCT      483
Arg Lys Ile Cys Thr Val Glu Leu Leu Thr Ala Lys Arg Val Gln Ser
                145                 150                 155

TTT CGG TCC ATA AGA GAA GAG GAG GTG GCA GAA CTA GTT AAA AAA ATA      531
Phe Arg Ser Ile Arg Glu Glu Glu Val Ala Glu Leu Val Lys Lys Ile
            160                 165                 170

GCT GCA ACT GCA AGT GAA GAA GGG GGG TCC ATT TTT AAT CTC ACC CAG      579
Ala Ala Thr Ala Ser Glu Glu Gly Gly Ser Ile Phe Asn Leu Thr Gln
        175                 180                 185

AGC ATT TAC TCA ATG ACT TTT GGG ATA GCG GCA CGA GCG GCT TTT GGT      627
Ser Ile Tyr Ser Met Thr Phe Gly Ile Ala Ala Arg Ala Ala Phe Gly
    190                 195                 200

AAA AAG AGC AGA TAC CAA CAA GTG TTC ATA TCA AAC ATG CAT AAA CAA      675
Lys Lys Ser Arg Tyr Gln Gln Val Phe Ile Ser Asn Met His Lys Gln
205                 210                 215                 220

TTG ATG CTT CTG GGA GGG TTT TCT GTT GCT GAT CTC TAT CCT TCT AGT      723
Leu Met Leu Leu Gly Gly Phe Ser Val Ala Asp Leu Tyr Pro Ser Ser
                225                 230                 235

AGA GTG TTT CAA ATG ATG GGG GCG ACG GGG AAA CTT GAA AAA GTG CAT      771
Arg Val Phe Gln Met Met Gly Ala Thr Gly Lys Leu Glu Lys Val His
            240                 245                 250

AGA GTG ACA GAT AGG GTG TTG CAA GAC ATC ATC GAC GAG CAC AAA AAT      819
Arg Val Thr Asp Arg Val Leu Gln Asp Ile Ile Asp Glu His Lys Asn
        255                 260                 265

AGA AAC AGA AGC AGC GAG GAG CGT GAA GCA GTG GAA GAT CTA GTT GAT      867
Arg Asn Arg Ser Ser Glu Glu Arg Glu Ala Val Glu Asp Leu Val Asp
    270                 275                 280

GTT CTT CTC AAG TTT CAA AAG GAA TCG GAA TTT CGC TTG ACT GAT GAC      915
Val Leu Leu Lys Phe Gln Lys Glu Ser Glu Phe Arg Leu Thr Asp Asp
285                 290                 295                 300

AAC ATT AAA GCC GTC ATC CAG GAC ATA TTC ATT GGT GGA GGC GAA ACA      963
Asn Ile Lys Ala Val Ile Gln Asp Ile Phe Ile Gly Gly Gly Glu Thr
                305                 310                 315

TCA TCT TCT GTT GTG GAA TGG GGG ATG TCA GAA TTG ATA AGA AAC CCG     1011
Ser Ser Ser Val Val Glu Trp Gly Met Ser Glu Leu Ile Arg Asn Pro
            320                 325                 330
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AGG GTG ATG GAA GAA GCA CAA GCA GAG GTG AGA AGA GTG TAT GAT AGC      1059
Arg Val Met Glu Glu Ala Gln Ala Glu Val Arg Arg Val Tyr Asp Ser
        335                 340                 345

AAG GGA TAT GTG GAT GAG ACA GAA TTG CAC CAA TTG ATA TAC TTA AAG      1107
Lys Gly Tyr Val Asp Glu Thr Glu Leu His Gln Leu Ile Tyr Leu Lys
    350                 355                 360

TCC ATC ATC AAA GAA ACC ATG AGG TTA CAT CCA CCT GTG CCA TTG TTA      1155
Ser Ile Ile Lys Glu Thr Met Arg Leu His Pro Pro Val Pro Leu Leu
365                 370                 375                 380

GTT CCT AGA GTA AGT AGA GAA AGG TGC CAA ATC AAT GGA TAT GAG ATA      1203
Val Pro Arg Val Ser Arg Glu Arg Cys Gln Ile Asn Gly Tyr Glu Ile
                385                 390                 395

CCC TCT AAG ACT AGG ATC ATT ATC AAT GCT TGG GCA ATT GGA AGG AAT      1251
Pro Ser Lys Thr Arg Ile Ile Ile Asn Ala Trp Ala Ile Gly Arg Asn
            400                 405                 410

CCT AAG TAT TGG GGT GAA ACT GAG AGT TTT AAA CCT GAG AGG TTT CTT      1299
Pro Lys Tyr Trp Gly Glu Thr Glu Ser Phe Lys Pro Glu Arg Phe Leu
        415                 420                 425

AAT AGC TCC ATT GAT TTT AGG GGC ACA GAC TTT GAA TTT ATC CCA TTT      1347
Asn Ser Ser Ile Asp Phe Arg Gly Thr Asp Phe Glu Phe Ile Pro Phe
    430                 435                 440

GGT GCT GGA AGG AGG ATC TGC CCC GGC ATT ACA TTT GCC ATA CCC AAC      1395
Gly Ala Gly Arg Arg Ile Cys Pro Gly Ile Thr Phe Ala Ile Pro Asn
445                 450                 455                 460

ATT GAG TTG CCA CTT GCT CAG TTA CTT TAC CAC TTT GAT TGG AAG CTT      1443
Ile Glu Leu Pro Leu Ala Gln Leu Leu Tyr His Phe Asp Trp Lys Leu
                465                 470                 475

CCC AAT AAA ATG AAG AAT GAA GAA CTT GAC ATG ACG GAG TCA AAT GGA      1491
Pro Asn Lys Met Lys Asn Glu Glu Leu Asp Met Thr Glu Ser Asn Gly
            480                 485                 490

ATT ACT TTA CGA AGA CAA AAT GAC CTC TGC TTG ATT CCC ATT ACT CGT      1539
Ile Thr Leu Arg Arg Gln Asn Asp Leu Cys Leu Ile Pro Ile Thr Arg
        495                 500                 505

CTA CCT TAAAATGTAT GAACAATTAA TGTCATAAAC TATTTAAGTT TTATCTTTTA      1595
Leu Pro
    510

CTACTTCCAG CATTTCGTAA TTGGACAATG ACTATGATTA ACTTAAGTTA CTTCCTTATG   1655

ATTAACTTGA CATATGAATG AACATTTCTA AGATAA                             1691
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 510 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Val Met Glu Leu His Asn His Thr Pro Phe Ser Ile Tyr Phe Ile
1 5 10 15

Thr Ser Ile Leu Phe Ile Phe Phe Val Phe Phe Lys Leu Val Gln Arg
20 25 30

Ser Asp Ser Lys Thr Ser Ser Thr Cys Lys Leu Pro Pro Gly Pro Arg
35 40 45

Thr Leu Pro Leu Ile Gly Asn Ile His Gln Ile Val Gly Ser Leu Pro
50 55 60

Val His Tyr Tyr Leu Lys Asn Leu Ala Asp Lys Tyr Gly Pro Leu Met
65 70 75 80

His Leu Lys Leu Gly Glu Val Ser Asn Ile Ile Val Thr Ser Pro Glu
85 90 95

Met Ala Gln Glu Ile Met Lys Thr His Asp Leu Asn Phe Ser Asp Arg
100 105 110

Pro Asp Phe Val Leu Ser Arg Ile Val Ser Tyr Asn Gly Ser Gly Ile
115 120 125

Val Phe Ser Gln His Gly Asp Tyr Trp Arg Gln Leu Arg Lys Ile Cys
130 135 140

Thr Val Glu Leu Leu Thr Ala Lys Arg Val Gln Ser Phe Arg Ser Ile
145 150 155 160

Arg Glu Glu Glu Val Ala Glu Leu Val Lys Lys Ile Ala Ala Thr Ala
165 170 175

Ser Glu Glu Gly Gly Ser Ile Phe Asn Leu Thr Gln Ser Ile Tyr Ser
180 185 190

Met Thr Phe Gly Ile Ala Ala Arg Ala Ala Phe Gly Lys Lys Ser Arg
195 200 205

Tyr Gln Gln Val Phe Ile Ser Asn Met His Lys Gln Leu Met Leu Leu
210 215 220

Gly Gly Phe Ser Val Ala Asp Leu Tyr Pro Ser Ser Arg Val Phe Gln
225 230 235 240

Met Met Gly Ala Thr Gly Lys Leu Glu Lys Val His Arg Val Thr Asp
245 250 255

Arg Val Leu Gln Asp Ile Ile Asp Glu His Lys Asn Arg Asn Arg Ser
260 265 270

Ser Glu Glu Arg Glu Ala Val Glu Asp Leu Val Asp Val Leu Leu Lys
275 280 285

Phe Gln Lys Glu Ser Glu Phe Arg Leu Thr Asp Asp Asn Ile Lys Ala

290 295 300

Val Ile Gln Asp Ile Phe Ile Gly Gly Gly Glu Thr Ser Ser Ser Val
305 310 315 320

Val Glu Trp Gly Met Ser Glu Leu Ile Arg Asn Pro Arg Val Met Glu
325 330 335

Glu Ala Gln Ala Glu Val Arg Arg Val Tyr Asp Ser Lys Gly Tyr Val
340 345 350

Asp Glu Thr Glu Leu His Gln Leu Ile Tyr Leu Lys Ser Ile Ile Lys
355 360 365

Glu Thr Met Arg Leu His Pro Pro Val Pro Leu Leu Val Pro Arg Val
370 375 380

Ser Arg Glu Arg Cys Gln Ile Asn Gly Tyr Glu Ile Pro Ser Lys Thr
385 390 395 400

Arg Ile Ile Ile Asn Ala Trp Ala Ile Gly Arg Asn Pro Lys Tyr Trp
405 410 415

Gly Glu Thr Glu Ser Phe Lys Pro Glu Arg Phe Leu Asn Ser Ser Ile
420 425 430

Asp Phe Arg Gly Thr Asp Phe Glu Phe Ile Pro Phe Gly Ala Gly Arg
435 440 445

Arg Ile Cys Pro Gly Ile Thr Phe Ala Ile Pro Asn Ile Glu Leu Pro
450 455 460

Leu Ala Gln Leu Leu Tyr His Phe Asp Trp Lys Leu Pro Asn Lys Met
465 470 475 480

Lys Asn Glu Glu Leu Asp Met Thr Glu Ser Asn Gly Ile Thr Leu Arg
485 490 495

Arg Gln Asn Asp Leu Cys Leu Ile Pro Ile Thr Arg Leu Pro
500 505 510

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1644 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 4..1542

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AAA ATG GCC ACT CTT TCC TCC TAC GAC CAC TTC ATC TTC ACT GCC TTA 48

```
    Met Ala Thr Leu Ser Ser Tyr Asp His Phe Ile Phe Thr Ala Leu
      1               5                  10                  15

GCT TTC TTC ATA TCT GGC CTA ATT TTC TTC CTC AAA CAG AAA TCC AAA          96
Ala Phe Phe Ile Ser Gly Leu Ile Phe Phe Leu Lys Gln Lys Ser Lys
                20                  25                  30

TCC AAA AAG TTC AAC CTC CCT CCA GGA CCC CCC GGG TGG CCT ATT GTT         144
Ser Lys Lys Phe Asn Leu Pro Pro Gly Pro Pro Gly Trp Pro Ile Val
            35                  40                  45

GGG AAC CTC TTC CAA GTT GCT CGT TCT GGG AAA CCT TTC TTT GAG TAT         192
Gly Asn Leu Phe Gln Val Ala Arg Ser Gly Lys Pro Phe Phe Glu Tyr
        50                  55                  60

GTG AAC GAT GTG AGA CTC AAA TAT GGC TCA ATC TTC ACC CTC AAG ATG         240
Val Asn Asp Val Arg Leu Lys Tyr Gly Ser Ile Phe Thr Leu Lys Met
    65                  70                  75

GGA ACA AGG ACC ATG ATC ATC CTC ACC GAC GCA AAA CTG GTC CAC GAG         288
Gly Thr Arg Thr Met Ile Ile Leu Thr Asp Ala Lys Leu Val His Glu
80                  85                  90                  95

GCC ATG ATC CAA AAG GGT GCA ACC TAC GCC ACC AGG CCC CCC GAG AAC         336
Ala Met Ile Gln Lys Gly Ala Thr Tyr Ala Thr Arg Pro Pro Glu Asn
                100                 105                 110

CCC ACC AGA ACC ATC TTC AGT GAA AAC AAG TTC ACC GTG AAT GCA GCG         384
Pro Thr Arg Thr Ile Phe Ser Glu Asn Lys Phe Thr Val Asn Ala Ala
            115                 120                 125

ACC TAT GGC CCC GTG TGG AAG TCG CTG AGG AGG AAC ATG GTG CAG AAC         432
Thr Tyr Gly Pro Val Trp Lys Ser Leu Arg Arg Asn Met Val Gln Asn
        130                 135                 140

ATG CTC AGC TCA ACA AGA CTT AAG GAG TTT CGC AGT GTT CGG GAC AAT         480
Met Leu Ser Ser Thr Arg Leu Lys Glu Phe Arg Ser Val Arg Asp Asn
    145                 150                 155

GCG ATG GAC AAG CTC ATC AAC AGA CTC AAG GAC GAG GCC GAG AAG AAT         528
Ala Met Asp Lys Leu Ile Asn Arg Leu Lys Asp Glu Ala Glu Lys Asn
160                 165                 170                 175

AAC GGC GTG GTT TGG GTG CTC AAG GAT GCC AGG TTT GCT GTT TTT TGC         576
Asn Gly Val Val Trp Val Leu Lys Asp Ala Arg Phe Ala Val Phe Cys
                180                 185                 190

ATA CTT GTG GCT ATG TGT TTT GGT CTT GAG ATG GAT GAG GAG ACA GTG         624
Ile Leu Val Ala Met Cys Phe Gly Leu Glu Met Asp Glu Glu Thr Val
            195                 200                 205

GAG AGA ATA GAT CAG GTT ATG AAG AGT GTT CTC ATC ACT TTG GAC CCG         672
Glu Arg Ile Asp Gln Val Met Lys Ser Val Leu Ile Thr Leu Asp Pro
        210                 215                 220

AGA ATT GAT GAC TAT CTT CCA ATT CTA AGC CCC TTT TTC TCA AAG CAA         720
Arg Ile Asp Asp Tyr Leu Pro Ile Leu Ser Pro Phe Phe Ser Lys Gln
    225                 230                 235
```

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AGA AAG AAA GCC TTG GAG GTT CGC AGA GAA CAG GTT GAG TTC TTA GTT      768
Arg Lys Lys Ala Leu Glu Val Arg Arg Glu Gln Val Glu Phe Leu Val
240                 245                 250                 255

CCA ATT ATA GAA CAA AGA AGA AGA GCA ATT CAA AAC CCT GGG TCA GAT      816
Pro Ile Ile Glu Gln Arg Arg Arg Ala Ile Gln Asn Pro Gly Ser Asp
                260                 265                 270

CAC ACC GCC ACA ACG TTT TCC TAC CTA GAC ACA CTT TTT GAC CTC AAA      864
His Thr Ala Thr Thr Phe Ser Tyr Leu Asp Thr Leu Phe Asp Leu Lys
            275                 280                 285

GTT GAA GGG AAG AAA TCA GCA CCC TCT GAT GCA GAA TTG GTG TCT TTA      912
Val Glu Gly Lys Lys Ser Ala Pro Ser Asp Ala Glu Leu Val Ser Leu
        290                 295                 300

TGC TCA GAG TTT CTT AAC GGT GGC ACA GAC ACA ACA GCA ACA GCG GTT      960
Cys Ser Glu Phe Leu Asn Gly Gly Thr Asp Thr Thr Ala Thr Ala Val
    305                 310                 315

GAG TGG GGC ATA GCA CAG CTC ATA GCG AAC CCT AAC GTT CAG ACA AAG     1008
Glu Trp Gly Ile Ala Gln Leu Ile Ala Asn Pro Asn Val Gln Thr Lys
320                 325                 330                 335

CTG TAC GAG GAA ATA AAG AGA ACG GTG GGA GAG AAG AAG GTG GAT GAA     1056
Leu Tyr Glu Glu Ile Lys Arg Thr Val Gly Glu Lys Lys Val Asp Glu
                340                 345                 350

AAG GAC GTT GAG AAA ATG CCA TAC CTA CAC GCT GTG GTG AAG GAG CTT     1104
Lys Asp Val Glu Lys Met Pro Tyr Leu His Ala Val Val Lys Glu Leu
            355                 360                 365

CTA AGA AAG CAC CCT CCA ACA CAC TTT GTG CTA ACA CAT GCT GTG ACT     1152
Leu Arg Lys His Pro Pro Thr His Phe Val Leu Thr His Ala Val Thr
        370                 375                 380

GAG CCC ACC ACT TTG GGA GGG TAT GAC ATA CCA ATT GAT GCA AAT GTT     1200
Glu Pro Thr Thr Leu Gly Gly Tyr Asp Ile Pro Ile Asp Ala Asn Val
    385                 390                 395

GAG GTG TAC ACA CCA GCC ATT GCT GAG GAC CCC AAA AAT TGG TTA AAC     1248
Glu Val Tyr Thr Pro Ala Ile Ala Glu Asp Pro Lys Asn Trp Leu Asn
400                 405                 410                 415

CCT GAG AAG TTT GAC CCT GAG AGA TTC ATC TCT GGG GGT GAG GAA GCA     1296
Pro Glu Lys Phe Asp Pro Glu Arg Phe Ile Ser Gly Gly Glu Glu Ala
                420                 425                 430

GAC ATA ACT GGG GTC ACA GGG GTG AAG ATG ATG CCA TTT GGG GTT GGG     1344
Asp Ile Thr Gly Val Thr Gly Val Lys Met Met Pro Phe Gly Val Gly
            435                 440                 445

AGA AGG ATT TGC CCT GGC TTG GCT ATG GCC ACA GTG CAT ATT CAC CTC     1392
Arg Arg Ile Cys Pro Gly Leu Ala Met Ala Thr Val His Ile His Leu
        450                 455                 460

ATG ATG GCA AGG ATG GTG CAG GAG TTT GAG TGG GGT GCA TAC CCT CCA     1440
Met Met Ala Arg Met Val Gln Glu Phe Glu Trp Gly Ala Tyr Pro Pro
    465                 470                 475
```

```
GAG AAG AAG ATG GAT TTC ACT GGC AAG TGG GAG TTC ACT GTG GTC ATG      1488
Glu Lys Lys Met Asp Phe Thr Gly Lys Trp Glu Phe Thr Val Val Met
480                 485                 490                 495

AAG GAG TCT CTA AGA GCA ACC ATC AAA CCA AGA GGA GGA GAA AAA GTG      1536
Lys Glu Ser Leu Arg Ala Thr Ile Lys Pro Arg Gly Gly Glu Lys Val
                500                 505                 510

AAG TTG TAAAATTTTC CTGCTTCTAT TCTTCTGGGT TTTAAATTTC ACAGACAACA      1592
Lys Leu

TAAATATTAT TGCTATTATC ATCATCATAT ATGTATACAT CATCATGGTT AC            1644
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 513 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Ala Thr Leu Ser Ser Tyr Asp His Phe Ile Phe Thr Ala Leu Ala
 1               5                  10                  15

Phe Phe Ile Ser Gly Leu Ile Phe Phe Leu Lys Gln Lys Ser Lys Ser
            20                  25                  30

Lys Lys Phe Asn Leu Pro Pro Gly Pro Pro Gly Trp Pro Ile Val Gly
        35                  40                  45

Asn Leu Phe Gln Val Ala Arg Ser Gly Lys Pro Phe Phe Glu Tyr Val
    50                  55                  60

Asn Asp Val Arg Leu Lys Tyr Gly Ser Ile Phe Thr Leu Lys Met Gly
65                  70                  75                  80

Thr Arg Thr Met Ile Ile Leu Thr Asp Ala Lys Leu Val His Glu Ala
                85                  90                  95

Met Ile Gln Lys Gly Ala Thr Tyr Ala Thr Arg Pro Pro Glu Asn Pro
            100                 105                 110

Thr Arg Thr Ile Phe Ser Glu Asn Lys Phe Thr Val Asn Ala Ala Thr
        115                 120                 125

Tyr Gly Pro Val Trp Lys Ser Leu Arg Arg Asn Met Val Gln Asn Met
    130                 135                 140

Leu Ser Ser Thr Arg Leu Lys Glu Phe Arg Ser Val Arg Asp Asn Ala
145                 150                 155                 160

Met Asp Lys Leu Ile Asn Arg Leu Lys Asp Glu Ala Glu Lys Asn Asn
                165                 170                 175
```

Gly Val Val Trp Val Leu Lys Asp Ala Arg Phe Ala Val Phe Cys Ile
180 185 190

Leu Val Ala Met Cys Phe Gly Leu Glu Met Asp Glu Glu Thr Val Glu
195 200 205

Arg Ile Asp Gln Val Met Lys Ser Val Leu Ile Thr Leu Asp Pro Arg
210 215 220

Ile Asp Asp Tyr Leu Pro Ile Leu Ser Pro Phe Phe Ser Lys Gln Arg
225 230 235 240

Lys Lys Ala Leu Glu Val Arg Arg Glu Gln Val Glu Phe Leu Val Pro
245 250 255

Ile Ile Glu Gln Arg Arg Arg Ala Ile Gln Asn Pro Gly Ser Asp His
260 265 270

Thr Ala Thr Thr Phe Ser Tyr Leu Asp Thr Leu Phe Asp Leu Lys Val
275 280 285

Glu Gly Lys Lys Ser Ala Pro Ser Asp Ala Glu Leu Val Ser Leu Cys
290 295 300

Ser Glu Phe Leu Asn Gly Gly Thr Asp Thr Thr Ala Thr Ala Val Glu
305 310 315 320

Trp Gly Ile Ala Gln Leu Ile Ala Asn Pro Asn Val Gln Thr Lys Leu
325 330 335

Tyr Glu Glu Ile Lys Arg Thr Val Gly Glu Lys Lys Val Asp Glu Lys
340 345 350

Asp Val Glu Lys Met Pro Tyr Leu His Ala Val Val Lys Glu Leu Leu
355 360 365

Arg Lys His Pro Pro Thr His Phe Val Leu Thr His Ala Val Thr Glu
370 375 380

Pro Thr Thr Leu Gly Gly Tyr Asp Ile Pro Ile Asp Ala Asn Val Glu
385 390 395 400

Val Tyr Thr Pro Ala Ile Ala Glu Asp Pro Lys Asn Trp Leu Asn Pro
405 410 415

Glu Lys Phe Asp Pro Glu Arg Phe Ile Ser Gly Gly Glu Glu Ala Asp
420 425 430

Ile Thr Gly Val Thr Gly Val Lys Met Met Pro Phe Gly Val Gly Arg
435 440 445

Arg Ile Cys Pro Gly Leu Ala Met Ala Thr Val His Ile His Leu Met
450 455 460

Met Ala Arg Met Val Gln Glu Phe Glu Trp Gly Ala Tyr Pro Pro Glu
465 470 475 480

Lys Lys Met Asp Phe Thr Gly Lys Trp Glu Phe Thr Val Val Met Lys
485 490 495

```
Glu Ser Leu Arg Ala Thr Ile Lys Pro Arg Gly Gly Glu Lys Val Lys
          500                 505                 510

Leu
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1611 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 20..1588

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
AAGCACTATC CCTCCCACC ATG ACA AGC CAC ATT GAC GAC AAC CTC TGG ATA      52
                     Met Thr Ser His Ile Asp Asp Asn Leu Trp Ile
                       1               5                  10

ATA GCC CTG ACC TCG AAA TGC ACC CAA GAA AAC CTT GCA TGG GTC CTT     100
Ile Ala Leu Thr Ser Lys Cys Thr Gln Glu Asn Leu Ala Trp Val Leu
            15                  20                  25

TTG ATC ATG GGC TCA CTC TGG TTA ACC ATG ACT TTC TAT TAC TGG TCA     148
Leu Ile Met Gly Ser Leu Trp Leu Thr Met Thr Phe Tyr Tyr Trp Ser
        30                  35                  40

CAC CCC GGT GGT CCT GCC TGG GGC AAG TAC TAC ACC TAC TCT CCC CCC     196
His Pro Gly Gly Pro Ala Trp Gly Lys Tyr Tyr Thr Tyr Ser Pro Pro
    45                  50                  55

CTT TCA ATC ATT CCC GGT CCC AAA GGC TTC CCT CTT ATT GGA AGC ATG     244
Leu Ser Ile Ile Pro Gly Pro Lys Gly Phe Pro Leu Ile Gly Ser Met
60                  65                  70                  75

GGC CTC ATG ACT TCC CTG GCC CAT CAC CGT ATC GCA GCC GCG GCC GCC     292
Gly Leu Met Thr Ser Leu Ala His His Arg Ile Ala Ala Ala Ala Ala
                80                  85                  90

ACA TGC AGA GCC AAG CGC CTC ATG GCC TTT AGT CTC GGC GAC ACA CGT     340
Thr Cys Arg Ala Lys Arg Leu Met Ala Phe Ser Leu Gly Asp Thr Arg
            95                  100                 105

GTC ATC GTC ACG TGC CAC CCC GAC GTG GCC AAG GAG ATT CTC AAC AGC     388
Val Ile Val Thr Cys His Pro Asp Val Ala Lys Glu Ile Leu Asn Ser
        110                 115                 120

TCC GTC TTC GCC GAT CGT CCC GTC AAA GAA TCC GCA TAC AGC CTC ATG     436
Ser Val Phe Ala Asp Arg Pro Val Lys Glu Ser Ala Tyr Ser Leu Met
    125                 130                 135
```

```
TTT AAC CGC GCC ATC GGC TTC GCC TCT TAC GGA GTT TAC TGG CGA AGC          484
Phe Asn Arg Ala Ile Gly Phe Ala Ser Tyr Gly Val Tyr Trp Arg Ser
140                 145                 150                 155

CTC AGG AGA ATC GCC TCT AAT CAC CTC TTC TGC CCC CGC CAG ATA AAA          532
Leu Arg Arg Ile Ala Ser Asn His Leu Phe Cys Pro Arg Gln Ile Lys
                160                 165                 170

GCC TCT GAG CTC CAA CGC TCT CAA ATC GCC GCC CAA ATG GTT CAC ATC          580
Ala Ser Glu Leu Gln Arg Ser Gln Ile Ala Ala Gln Met Val His Ile
            175                 180                 185

CTA AAT AAC AAG CGC CAC CGC AGC TTA CGT GTT CGC CAA GTG CTG AAA          628
Leu Asn Asn Lys Arg His Arg Ser Leu Arg Val Arg Gln Val Leu Lys
        190                 195                 200

AAG GCT TCG CTC AGT AAC ATG ATG TGC TCC GTG TTT GGA CAA GAG TAT          676
Lys Ala Ser Leu Ser Asn Met Met Cys Ser Val Phe Gly Gln Glu Tyr
    205                 210                 215

AAG CTG CAC GAC CCA AAC AGC GGA ATG GAA GAC CTT GGA ATA TTA GTG          724
Lys Leu His Asp Pro Asn Ser Gly Met Glu Asp Leu Gly Ile Leu Val
220                 225                 230                 235

GAC CAA GGT TAT GAC CTG TTG GGC CTG TTT AAT TGG GCC GAC CAC CTT          772
Asp Gln Gly Tyr Asp Leu Leu Gly Leu Phe Asn Trp Ala Asp His Leu
                240                 245                 250

CCT TTT CTT GCA CAT TTC GAC GCC CAA AAT ATC CGG TTC AGG TGC TCC          820
Pro Phe Leu Ala His Phe Asp Ala Gln Asn Ile Arg Phe Arg Cys Ser
            255                 260                 265

AAC CTC GTC CCC ATG GTG AAC CGT TTC GTC GGC ACA ATC ATC GCT GAA          868
Asn Leu Val Pro Met Val Asn Arg Phe Val Gly Thr Ile Ile Ala Glu
        270                 275                 280

CAC CGA GCT AGT AAA ACC GAA ACC AAT CGT GAT TTT GTT GAC GTC TTG          916
His Arg Ala Ser Lys Thr Glu Thr Asn Arg Asp Phe Val Asp Val Leu
    285                 290                 295

CTC TCT CTC CCG GAA CCT GAT CAA TTA TCA GAC TCC GAC ATG ATC GCT          964
Leu Ser Leu Pro Glu Pro Asp Gln Leu Ser Asp Ser Asp Met Ile Ala
300                 305                 310                 315

GTA CTT TGG GAA ATG ATA TTC AGA GGA ACG GAC ACG GTA GCG GTT TTG         1012
Val Leu Trp Glu Met Ile Phe Arg Gly Thr Asp Thr Val Ala Val Leu
                320                 325                 330

ATA GAG TGG ATA CTC GCG AGG ATG GCG CTT CAT CCT CAT GTG CAG TCC         1060
Ile Glu Trp Ile Leu Ala Arg Met Ala Leu His Pro His Val Gln Ser
            335                 340                 345

AAA GTT CAA GAG GAG CTA GAT GCA GTT GTC GGA AAA GCA CGC GCC GTC         1108
Lys Val Gln Glu Glu Leu Asp Ala Val Val Gly Lys Ala Arg Ala Val
        350                 355                 360

GCA GAG GAT GAC GTG GCA GTG ATG ACG TAC CTA CCA GCG GTG GTG AAG         1156
Ala Glu Asp Asp Val Ala Val Met Thr Tyr Leu Pro Ala Val Val Lys
    365                 370                 375
```

```
GAG GTG CTG CGG CTG CAC CCG CCG GGC CCA CTT CTA TCA TGG GCC CGC      1204
Glu Val Leu Arg Leu His Pro Pro Gly Pro Leu Leu Ser Trp Ala Arg
380                 385                 390                 395

TTG TCC ATC AAT GAT ACG ACC ATT GAT GGG TAT CAC GTA CCT GCG GGG      1252
Leu Ser Ile Asn Asp Thr Thr Ile Asp Gly Tyr His Val Pro Ala Gly
            400                 405                 410

ACC ACT GCT ATG GTC AAC ACG TGG GCT ATT TGC AGG GAC CCA CAC GTG      1300
Thr Thr Ala Met Val Asn Thr Trp Ala Ile Cys Arg Asp Pro His Val
        415                 420                 425

TGG AAG GAC CCA CTC GAA TTT ATG CCC GAG AGG TTT GTC ACT GCG GGT      1348
Trp Lys Asp Pro Leu Glu Phe Met Pro Glu Arg Phe Val Thr Ala Gly
    430                 435                 440

GGA GAT GCC GAA TTT TCG ATA CTC GGG TCG GAT CCA AGA CTT GCT CCA      1396
Gly Asp Ala Glu Phe Ser Ile Leu Gly Ser Asp Pro Arg Leu Ala Pro
445                 450                 455

TTT GGG TCG GGT AGG AGA GCG TGC CCA GGG AAG ACT CTT GGA TGG GCT      1444
Phe Gly Ser Gly Arg Arg Ala Cys Pro Gly Lys Thr Leu Gly Trp Ala
460                 465                 470                 475

ACG GTG AAC TTT TGG GTG GCG TCG CTC TTG CAT GAG TTC GAA TGG GTA      1492
Thr Val Asn Phe Trp Val Ala Ser Leu Leu His Glu Phe Glu Trp Val
            480                 485                 490

CCG TCT GAT GAG AAG GGT GTT GAT CTG ACG GAG GTG CTG AAG CTC TCT      1540
Pro Ser Asp Glu Lys Gly Val Asp Leu Thr Glu Val Leu Lys Leu Ser
        495                 500                 505

AGT GAA ATG GCT AAC CCT CTC ACC GTC AAA GTG CGC CCC AGG CGT GGA      1588
Ser Glu Met Ala Asn Pro Leu Thr Val Lys Val Arg Pro Arg Arg Gly
    510                 515                 520

TAAGAGAGAG TTGAAGCTTT TAT                                           1611
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 523 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Met Thr Ser His Ile Asp Asp Asn Leu Trp Ile Ile Ala Leu Thr Ser
 1               5                  10                  15

Lys Cys Thr Gln Glu Asn Leu Ala Trp Val Leu Leu Ile Met Gly Ser
            20                  25                  30

Leu Trp Leu Thr Met Thr Phe Tyr Tyr Trp Ser His Pro Gly Gly Pro
        35                  40                  45
```

Ala Trp Gly Lys Tyr Tyr Thr Tyr Ser Pro Pro Leu Ser Ile Ile Pro
50 55 60

Gly Pro Lys Gly Phe Pro Leu Ile Gly Ser Met Gly Leu Met Thr Ser
65 70 75 80

Leu Ala His His Arg Ile Ala Ala Ala Ala Ala Thr Cys Arg Ala Lys
85 90 95

Arg Leu Met Ala Phe Ser Leu Gly Asp Thr Arg Val Ile Val Thr Cys
100 105 110

His Pro Asp Val Ala Lys Glu Ile Leu Asn Ser Ser Val Phe Ala Asp
115 120 125

Arg Pro Val Lys Glu Ser Ala Tyr Ser Leu Met Phe Asn Arg Ala Ile
130 135 140

Gly Phe Ala Ser Tyr Gly Val Tyr Trp Arg Ser Leu Arg Arg Ile Ala
145 150 155 160

Ser Asn His Leu Phe Cys Pro Arg Gln Ile Lys Ala Ser Glu Leu Gln
165 170 175

Arg Ser Gln Ile Ala Ala Gln Met Val His Ile Leu Asn Asn Lys Arg
180 185 190

His Arg Ser Leu Arg Val Arg Gln Val Leu Lys Lys Ala Ser Leu Ser
195 200 205

Asn Met Met Cys Ser Val Phe Gly Gln Glu Tyr Lys Leu His Asp Pro
210 215 220

Asn Ser Gly Met Glu Asp Leu Gly Ile Leu Val Asp Gln Gly Tyr Asp
225 230 235 240

Leu Leu Gly Leu Phe Asn Trp Ala Asp His Leu Pro Phe Leu Ala His
245 250 255

Phe Asp Ala Gln Asn Ile Arg Phe Arg Cys Ser Asn Leu Val Pro Met
260 265 270

Val Asn Arg Phe Val Gly Thr Ile Ile Ala Glu His Arg Ala Ser Lys
275 280 285

Thr Glu Thr Asn Arg Asp Phe Val Asp Val Leu Leu Ser Leu Pro Glu
290 295 300

Pro Asp Gln Leu Ser Asp Ser Asp Met Ile Ala Val Leu Trp Glu Met
305 310 315 320

Ile Phe Arg Gly Thr Asp Thr Val Ala Val Leu Ile Glu Trp Ile Leu
325 330 335

Ala Arg Met Ala Leu His Pro His Val Gln Ser Lys Val Gln Glu Glu
340 345 350

Leu Asp Ala Val Val Gly Lys Ala Arg Ala Val Ala Glu Asp Asp Val
355 360 365

```
Ala Val Met Thr Tyr Leu Pro Ala Val Val Lys Glu Val Leu Arg Leu
    370                 375                 380

His Pro Pro Gly Pro Leu Leu Ser Trp Ala Arg Leu Ser Ile Asn Asp
385                 390                 395                 400

Thr Thr Ile Asp Gly Tyr His Val Pro Ala Gly Thr Thr Ala Met Val
                405                 410                 415

Asn Thr Trp Ala Ile Cys Arg Asp Pro His Val Trp Lys Asp Pro Leu
            420                 425                 430

Glu Phe Met Pro Glu Arg Phe Val Thr Ala Gly Gly Asp Ala Glu Phe
        435                 440                 445

Ser Ile Leu Gly Ser Asp Pro Arg Leu Ala Pro Phe Gly Ser Gly Arg
    450                 455                 460

Arg Ala Cys Pro Gly Lys Thr Leu Gly Trp Ala Thr Val Asn Phe Trp
465                 470                 475                 480

Val Ala Ser Leu Leu His Glu Phe Glu Trp Val Pro Ser Asp Glu Lys
                485                 490                 495

Gly Val Asp Leu Thr Glu Val Leu Lys Leu Ser Ser Glu Met Ala Asn
            500                 505                 510

Pro Leu Thr Val Lys Val Arg Pro Arg Arg Gly
        515                 520
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1788 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 6..1601

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
GGGTC ATG GGC ATG GCC ATG GAT GCT TTC CAG CAC CAA ACT CTC ATT          47
      Met Gly Met Ala Met Asp Ala Phe Gln His Gln Thr Leu Ile
        1               5                  10

TCC ATC ATT CTG GCC ATG TTA GTA GGC GTG TTG ATT TAT GGC TTA AAG        95
Ser Ile Ile Leu Ala Met Leu Val Gly Val Leu Ile Tyr Gly Leu Lys
 15                  20                  25                  30

AGA ACA CAT AGT GGC CAT GGC AAG ATC TGT AGT GCA CCT CAA GCA GGA       143
Arg Thr His Ser Gly His Gly Lys Ile Cys Ser Ala Pro Gln Ala Gly
                35                  40                  45
```

```
GGA GCA TGG CCA ATT ATT GGC CAT TTA CAC CTC TTT GGG GGT CAT CAA      191
Gly Ala Trp Pro Ile Ile Gly His Leu His Leu Phe Gly Gly His Gln
            50                  55                  60

CAT ACT CAC AAA ACA CTT GGG ATA ATG GCA GAG AAA CAT GGA CCA ATT      239
His Thr His Lys Thr Leu Gly Ile Met Ala Glu Lys His Gly Pro Ile
        65                  70                  75

TTC ACA ATA AAG CTT GGT TCA TAC AAA GTT CTT GTA TTG AGT AGC TGG      287
Phe Thr Ile Lys Leu Gly Ser Tyr Lys Val Leu Val Leu Ser Ser Trp
    80                  85                  90

GAG ATG GCC AAG GAG TGT TTC ACT GTC CAT GAC AAA GCA TTT TCT ACC      335
Glu Met Ala Lys Glu Cys Phe Thr Val His Asp Lys Ala Phe Ser Thr
95                  100                 105                 110

AGA CCC TGT GTT GCA GCC TCA AAG CTA ATG GGC TAC AAC TAT GCC ATG      383
Arg Pro Cys Val Ala Ala Ser Lys Leu Met Gly Tyr Asn Tyr Ala Met
                115                 120                 125

TTT GGC TTC ACT CCT TAT GGT CCT TAT TGG CGT GAG ATA AGG AAA TTA      431
Phe Gly Phe Thr Pro Tyr Gly Pro Tyr Trp Arg Glu Ile Arg Lys Leu
            130                 135                 140

ACT ACT ATT CAG CTT CTA TCT AAC CAC CGG CTT GAA CTG CTG AAG AAC      479
Thr Thr Ile Gln Leu Leu Ser Asn His Arg Leu Glu Leu Leu Lys Asn
        145                 150                 155

ACA AGA ACA TCT GAG TCA GAA GTT GCA ATA AGA GAG CTT TAT AAG TTG      527
Thr Arg Thr Ser Glu Ser Glu Val Ala Ile Arg Glu Leu Tyr Lys Leu
    160                 165                 170

TGG TCT AGA GAA GGT TGT CCA AAG GGA GGG GTT TTG GTA GAT ATG AAG      575
Trp Ser Arg Glu Gly Cys Pro Lys Gly Gly Val Leu Val Asp Met Lys
175                 180                 185                 190

CAG TGG TTT GGG GAT TTA ACT CAT AAT ATT GTT CTG AGA ATG GTG AGA      623
Gln Trp Phe Gly Asp Leu Thr His Asn Ile Val Leu Arg Met Val Arg
                195                 200                 205

GGG AAG CCA TAC TAT GAT GGT GCT AGT GAT GAT TAT GCA GAA GGT GAA      671
Gly Lys Pro Tyr Tyr Asp Gly Ala Ser Asp Asp Tyr Ala Glu Gly Glu
            210                 215                 220

GCA AGA AGG TAC AAG AAA GTT ATG GGA GAG TGT GTG AGT TTG TTT GGG      719
Ala Arg Arg Tyr Lys Lys Val Met Gly Glu Cys Val Ser Leu Phe Gly
        225                 230                 235

GTG TTT GTG TTA TCT GAT GCT ATT CCA TTT CTG GGG TGG TTG GAC ATC      767
Val Phe Val Leu Ser Asp Ala Ile Pro Phe Leu Gly Trp Leu Asp Ile
    240                 245                 250

AAC GGA TAT GAA AAG GCC ATG AAG AGA ACT GCA AGT GAA TTG GAT CCT      815
Asn Gly Tyr Glu Lys Ala Met Lys Arg Thr Ala Ser Glu Leu Asp Pro
255                 260                 265                 270

CTG GTT GAA GGG TGG TTA GAG GAA CAC AAA AGG AAA AGA GCT TTC AAT      863
Leu Val Glu Gly Trp Leu Glu Glu His Lys Arg Lys Arg Ala Phe Asn
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                275                 280                 285

ATG GAT GCA AAA GAA GAA CAG GAT AAT TTC ATG GAT GTC ATG CTG AAT      911
Met Asp Ala Lys Glu Glu Gln Asp Asn Phe Met Asp Val Met Leu Asn
            290                 295                 300

GTT CTG AAA GAT GCA GAG ATT TCT GGT TAT GAT TCA GAT ACC ATC ATC      959
Val Leu Lys Asp Ala Glu Ile Ser Gly Tyr Asp Ser Asp Thr Ile Ile
        305                 310                 315

AAG GCT ACT TGT CTG AAT CTG ATT TTA GCA GGA AGC GAC ACC ACC ATG     1007
Lys Ala Thr Cys Leu Asn Leu Ile Leu Ala Gly Ser Asp Thr Thr Met
    320                 325                 330

ATT TCA CTA ACA TGG GTG CTA TCT CTG CTA CTT AAC CAT CAA ATG GAA     1055
Ile Ser Leu Thr Trp Val Leu Ser Leu Leu Leu Asn His Gln Met Glu
335                 340                 345                 350

CTA AAA AAA GTC CAA GAT GAA TTG GAC ACT TAT ATT GGG AAG GAC AGG     1103
Leu Lys Lys Val Gln Asp Glu Leu Asp Thr Tyr Ile Gly Lys Asp Arg
                355                 360                 365

AAG GTG GAA GAA TCT GAC ATA ACC AAG TTG GTG TAC CTC CAA GCC ATT     1151
Lys Val Glu Glu Ser Asp Ile Thr Lys Leu Val Tyr Leu Gln Ala Ile
            370                 375                 380

GTG AAG GAA ACA ATG CGG CTG TAT CCA CCA AGT CCT CTT ATC ACC CTT     1199
Val Lys Glu Thr Met Arg Leu Tyr Pro Pro Ser Pro Leu Ile Thr Leu
        385                 390                 395

CGT GCA GCC ATG GAA GAC TGC ACC TTC TCA GGT GGC TAT CAC ATT CCT     1247
Arg Ala Ala Met Glu Asp Cys Thr Phe Ser Gly Gly Tyr His Ile Pro
    400                 405                 410

GCT GGG ACA CGT TTA ATG GTG AAT GCT TGG AAG ATC CAC CGG GAT GGT     1295
Ala Gly Thr Arg Leu Met Val Asn Ala Trp Lys Ile His Arg Asp Gly
415                 420                 425                 430

CGT GTT TGG AGT GAT CCT CAT GAT TTC AAG CCT GGA AGG TTC TTG ACA     1343
Arg Val Trp Ser Asp Pro His Asp Phe Lys Pro Gly Arg Phe Leu Thr
                435                 440                 445

AGC CAC AAA GAT GTT GAT GTG AAG GGT CAG AAC TAT GAG CTC GTC CCT     1391
Ser His Lys Asp Val Asp Val Lys Gly Gln Asn Tyr Glu Leu Val Pro
            450                 455                 460

TTT GGT TCT GGA AGG AGA GCA TGC CCT GGA GCC TCG CTG GCT CTG CGT     1439
Phe Gly Ser Gly Arg Arg Ala Cys Pro Gly Ala Ser Leu Ala Leu Arg
        465                 470                 475

GTG GTG CAC TTG ACC ATG GCT AGA CTG TTA CAT TCT TTC AAT GTT GCT     1487
Val Val His Leu Thr Met Ala Arg Leu Leu His Ser Phe Asn Val Ala
    480                 485                 490

TCT CCT TCA AAT CAA GTT GTG GAC ATG ACA GAG AGC ATT GGA CTC ACA     1535
Ser Pro Ser Asn Gln Val Val Asp Met Thr Glu Ser Ile Gly Leu Thr
495                 500                 505                 510

AAT TTA AAA GCA ACC CCG CTT GAA ATT CTC CTA ACT CCA CGT CTA GAC     1583
```

```
Asn Leu Lys Ala Thr Pro Leu Glu Ile Leu Leu Thr Pro Arg Leu Asp
                515                 520                 525

ACC AAA CTT TAT GAG AAC TAGATTAAAT TAAGCTAGTT TTCTCCCAAA                1631
Thr Lys Leu Tyr Glu Asn
            530

TAAGGGGAGG GGTCCTCTAG GTCCTGAAAT CGGGTAATAA CAATAACATG GTTAATGCAG      1691

CTTCCATGTA GGATAATGAT TATTCACTCA TGGGTCACCT TTTAATGGAG CCTCAGTGTA      1751

TTATAATAAC TCCAAACTTG TGGGTCACAA TCCCCCC                                1788
```

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 532 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Met Gly Met Ala Met Asp Ala Phe Gln His Gln Thr Leu Ile Ser Ile
 1               5                  10                  15

Ile Leu Ala Met Leu Val Gly Val Leu Ile Tyr Gly Leu Lys Arg Thr
            20                  25                  30

His Ser Gly His Gly Lys Ile Cys Ser Ala Pro Gln Ala Gly Gly Ala
        35                  40                  45

Trp Pro Ile Ile Gly His Leu His Leu Phe Gly Gly His Gln His Thr
    50                  55                  60

His Lys Thr Leu Gly Ile Met Ala Glu Lys His Gly Pro Ile Phe Thr
65                  70                  75                  80

Ile Lys Leu Gly Ser Tyr Lys Val Leu Val Leu Ser Ser Trp Glu Met
                85                  90                  95

Ala Lys Glu Cys Phe Thr Val His Asp Lys Ala Phe Ser Thr Arg Pro
            100                 105                 110

Cys Val Ala Ala Ser Lys Leu Met Gly Tyr Asn Tyr Ala Met Phe Gly
        115                 120                 125

Phe Thr Pro Tyr Gly Pro Tyr Trp Arg Glu Ile Arg Lys Leu Thr Thr
    130                 135                 140

Ile Gln Leu Leu Ser Asn His Arg Leu Glu Leu Leu Lys Asn Thr Arg
145                 150                 155                 160

Thr Ser Glu Ser Glu Val Ala Ile Arg Glu Leu Tyr Lys Leu Trp Ser
                165                 170                 175

Arg Glu Gly Cys Pro Lys Gly Gly Val Leu Val Asp Met Lys Gln Trp
            180                 185                 190
```

Phe Gly Asp Leu Thr His Asn Ile Val Leu Arg Met Val Arg Gly Lys
195 200 205

Pro Tyr Tyr Asp Gly Ala Ser Asp Asp Tyr Ala Glu Gly Glu Ala Arg
210 215 220

Arg Tyr Lys Lys Val Met Gly Glu Cys Val Ser Leu Phe Gly Val Phe
225 230 235 240

Val Leu Ser Asp Ala Ile Pro Phe Leu Gly Trp Leu Asp Ile Asn Gly
245 250 255

Tyr Glu Lys Ala Met Lys Arg Thr Ala Ser Glu Leu Asp Pro Leu Val
260 265 270

Glu Gly Trp Leu Glu Glu His Lys Arg Lys Arg Ala Phe Asn Met Asp
275 280 285

Ala Lys Glu Glu Gln Asp Asn Phe Met Asp Val Met Leu Asn Val Leu
290 295 300

Lys Asp Ala Glu Ile Ser Gly Tyr Asp Ser Asp Thr Ile Ile Lys Ala
305 310 315 320

Thr Cys Leu Asn Leu Ile Leu Ala Gly Ser Asp Thr Thr Met Ile Ser
325 330 335

Leu Thr Trp Val Leu Ser Leu Leu Leu Asn His Gln Met Glu Leu Lys
340 345 350

Lys Val Gln Asp Glu Leu Asp Thr Tyr Ile Gly Lys Asp Arg Lys Val
355 360 365

Glu Glu Ser Asp Ile Thr Lys Leu Val Tyr Leu Gln Ala Ile Val Lys
370 375 380

Glu Thr Met Arg Leu Tyr Pro Pro Ser Pro Leu Ile Thr Leu Arg Ala
385 390 395 400

Ala Met Glu Asp Cys Thr Phe Ser Gly Gly Tyr His Ile Pro Ala Gly
405 410 415

Thr Arg Leu Met Val Asn Ala Trp Lys Ile His Arg Asp Gly Arg Val
420 425 430

Trp Ser Asp Pro His Asp Phe Lys Pro Gly Arg Phe Leu Thr Ser His
435 440 445

Lys Asp Val Asp Val Lys Gly Gln Asn Tyr Glu Leu Val Pro Phe Gly
450 455 460

Ser Gly Arg Arg Ala Cys Pro Gly Ala Ser Leu Ala Leu Arg Val Val
465 470 475 480

His Leu Thr Met Ala Arg Leu Leu His Ser Phe Asn Val Ala Ser Pro
485 490 495

Ser Asn Gln Val Val Asp Met Thr Glu Ser Ile Gly Leu Thr Asn Leu
500 505 510

```
Lys Ala Thr Pro Leu Glu Ile Leu Leu Thr Pro Arg Leu Asp Thr Lys
        515                 520                 525

Leu Tyr Glu Asn
    530
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1657 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1548

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
CTT GTT CTT CTT TCT CTA TTG TCT ATA GTC ATC TCC ATT GTT CTC TTC      48
Leu Val Leu Leu Ser Leu Leu Ser Ile Val Ile Ser Ile Val Leu Phe
  1               5                  10                  15

ATT ACC CAC ACA CAC AAA AGA AAC AAC ACT CCA AGA GGA CCA CCA GGT      96
Ile Thr His Thr His Lys Arg Asn Asn Thr Pro Arg Gly Pro Pro Gly
            20                  25                  30

CCT CCA CCT CTT CCT CTC ATC GGC AAC CTT CAC CAA CTC CAC AAC TCA     144
Pro Pro Pro Leu Pro Leu Ile Gly Asn Leu His Gln Leu His Asn Ser
        35                  40                  45

TCC CCA CAT CTC TGC CTA TGG CAA CTC GCC AAA CTC CAC GGT CCT CTC     192
Ser Pro His Leu Cys Leu Trp Gln Leu Ala Lys Leu His Gly Pro Leu
    50                  55                  60

ATG TCG TTT CGC CTC GGC GCC GTG CAA ACC GTC GTG GTT TCA TCG GCC     240
Met Ser Phe Arg Leu Gly Ala Val Gln Thr Val Val Val Ser Ser Ala
65                  70                  75                  80

AGA ATC GCC GAA CAA ATC TTG AAA ACC CAC GAC CTC AAC TTC GCT TCC     288
Arg Ile Ala Glu Gln Ile Leu Lys Thr His Asp Leu Asn Phe Ala Ser
                85                  90                  95

AGG CCT CTC TTC GTG GGC CCG AGA AAG CTC TCT TAC GAC GGG TTG GAC     336
Arg Pro Leu Phe Val Gly Pro Arg Lys Leu Ser Tyr Asp Gly Leu Asp
            100                 105                 110

ATG GGC TTC GCA CCG TAC GGC CCG TAC TGG AGA GAA ATG AAG AAA CTC     384
Met Gly Phe Ala Pro Tyr Gly Pro Tyr Trp Arg Glu Met Lys Lys Leu
        115                 120                 125

TGC ATC GTT CAC CTC TTC AGC GCG CAA CGC GTT CGG TCC TTT CGA CCA     432
Cys Ile Val His Leu Phe Ser Ala Gln Arg Val Arg Ser Phe Arg Pro
```

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    130                 135                 140

ATT CGA GAG AAC GAG GTT GCA AAA ATG GTT CGG AAA CTG TCG GAA CAC      480
Ile Arg Glu Asn Glu Val Ala Lys Met Val Arg Lys Leu Ser Glu His
145                 150                 155                 160

GAA GCT TCG GGT ACT GTC GTG AAC TTG ACC GAA ACT TTG ATG TCT TTC      528
Glu Ala Ser Gly Thr Val Val Asn Leu Thr Glu Thr Leu Met Ser Phe
                165                 170                 175

ACG AAC TCT TTG ATA TGC AGA ATC GCG TTG GGG AAA AGT TAC GGT TGT      576
Thr Asn Ser Leu Ile Cys Arg Ile Ala Leu Gly Lys Ser Tyr Gly Cys
            180                 185                 190

GAG TAC GAG GAA GTA GTT GTT GAT GAG GTA CTG GGA AAC CGG AGG AGC      624
Glu Tyr Glu Glu Val Val Val Asp Glu Val Leu Gly Asn Arg Arg Ser
        195                 200                 205

AGG TTG CAG GTT CTG CTC AAC GAG GCT CAA GCG TTG CTT TCG GAG TTT      672
Arg Leu Gln Val Leu Leu Asn Glu Ala Gln Ala Leu Leu Ser Glu Phe
    210                 215                 220

TTC TTT TCG GAT TAT TTT CCG CCT ATA GGA AAG TGG GTT GAT AGA GTG      720
Phe Phe Ser Asp Tyr Phe Pro Pro Ile Gly Lys Trp Val Asp Arg Val
225                 230                 235                 240

ACG GGA ATT CTA TCG CGG CTT GAT AAA ACG TTC AAG GAG TTG GAC GCG      768
Thr Gly Ile Leu Ser Arg Leu Asp Lys Thr Phe Lys Glu Leu Asp Ala
                245                 250                 255

TGC TAC GAA CGA TCA TCC TAT GAT CAC ATG GAT TCG GCA AAG AGT GGT      816
Cys Tyr Glu Arg Ser Ser Tyr Asp His Met Asp Ser Ala Lys Ser Gly
            260                 265                 270

AAA AAA GAT AAT GAC AAC AAA GAA GTC AAA GAT ATT ATT GAT ATT CTT      864
Lys Lys Asp Asn Asp Asn Lys Glu Val Lys Asp Ile Ile Asp Ile Leu
        275                 280                 285

CTC CAG CTA CTT GAT GAT CGT TCC TTC ACC TTT GAT CTC ACT CTC GAC      912
Leu Gln Leu Leu Asp Asp Arg Ser Phe Thr Phe Asp Leu Thr Leu Asp
    290                 295                 300

CAC ATA AAA GCC GTG CTC ATG AAC ATC TTT ATA GCA GGA ACA GAC CCG      960
His Ile Lys Ala Val Leu Met Asn Ile Phe Ile Ala Gly Thr Asp Pro
305                 310                 315                 320

AGT TCC GCG ACA ATA GTT TGG GCA ATG AAT GCA CTG TTG AAG AAT CCC     1008
Ser Ser Ala Thr Ile Val Trp Ala Met Asn Ala Leu Leu Lys Asn Pro
                325                 330                 335

AAT GTG ATG AGC AAG GTT CAA GGA GAA GTG AGA AAT CTA TTC GGT GAC     1056
Asn Val Met Ser Lys Val Gln Gly Glu Val Arg Asn Leu Phe Gly Asp
            340                 345                 350

AAA GAT TTC ATA AAC GAA GAT GAT GTC GAA AGC CTT CCT TAT CTC AAA     1104
Lys Asp Phe Ile Asn Glu Asp Asp Val Glu Ser Leu Pro Tyr Leu Lys
        355                 360                 365

GCA GTG GTG AAG GAG ACA TTA AGA TTA TTC CCA CCT TCA CCA CTA CTT     1152
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```
Ala Val Val Lys Glu Thr Leu Arg Leu Phe Pro Pro Ser Pro Leu Leu
    370                 375                 380

TTG CCA AGG GTA ACA ATG GAA ACA TGC AAC ATA GAA GGG TAC GAA ATT      1200
Leu Pro Arg Val Thr Met Glu Thr Cys Asn Ile Glu Gly Tyr Glu Ile
385                 390                 395                 400

CAA GCC AAA ACT ATA GTG CAT GTT AAT GCA TGG GCC ATA GCA AGG GAC      1248
Gln Ala Lys Thr Ile Val His Val Asn Ala Trp Ala Ile Ala Arg Asp
                405                 410                 415

CCT GAG AAT TGG GAA GAG CCT GAG AAA TTT TTC CCC GAA AGG TTC CTT      1296
Pro Glu Asn Trp Glu Glu Pro Glu Lys Phe Phe Pro Glu Arg Phe Leu
            420                 425                 430

GAG AGT TCG ATG GAG TTA AAG GGG AAT GAT GAG TTT AAG GTG ATC CCG      1344
Glu Ser Ser Met Glu Leu Lys Gly Asn Asp Glu Phe Lys Val Ile Pro
        435                 440                 445

TTT GGT TCT GGA AGG AGA ATG TGT CCT GCG AAG CAC ATG GGA ATT ATG      1392
Phe Gly Ser Gly Arg Arg Met Cys Pro Ala Lys His Met Gly Ile Met
    450                 455                 460

AAT GTT GAG CTT TCT CTT GCT AAT CTC ATT CAC ACG TTT GAT TGG GAA      1440
Asn Val Glu Leu Ser Leu Ala Asn Leu Ile His Thr Phe Asp Trp Glu
465                 470                 475                 480

GTG GCT AAA GGG TTC GAC AAG GAA GAA ATG TTG GAC ACG CAA ATG AAA      1488
Val Ala Lys Gly Phe Asp Lys Glu Glu Met Leu Asp Thr Gln Met Lys
                485                 490                 495

CCA GGA ATA ACG ATG CAC AAG AAA AGT GAT CTT TAC CTA GTG GCA AAG      1536
Pro Gly Ile Thr Met His Lys Lys Ser Asp Leu Tyr Leu Val Ala Lys
            500                 505                 510

AAA CCG ACA ACG TAGCACACGT TGGTACATTC ACTATAACAC ACAAGAAAGT          1588
Lys Pro Thr Thr
        515

TGATAATGAC TTGTGTATGC AACTATGCTC TATGCACTAT GCACTATGTT TATTGACCAT    1648

TAATTACTG                                                           1657
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 516 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Leu Val Leu Leu Ser Leu Leu Ser Ile Val Ile Ser Ile Val Leu Phe
  1               5                  10                  15

Ile Thr His Thr His Lys Arg Asn Asn Thr Pro Arg Gly Pro Pro Gly
            20                  25                  30
```

Pro Pro Pro Leu Pro Leu Ile Gly Asn Leu His Gln Leu His Asn Ser
35 40 45

Ser Pro His Leu Cys Leu Trp Gln Leu Ala Lys Leu His Gly Pro Leu
50 55 60

Met Ser Phe Arg Leu Gly Ala Val Gln Thr Val Val Val Ser Ser Ala
65 70 75 80

Arg Ile Ala Glu Gln Ile Leu Lys Thr His Asp Leu Asn Phe Ala Ser
85 90 95

Arg Pro Leu Phe Val Gly Pro Arg Lys Leu Ser Tyr Asp Gly Leu Asp
100 105 110

Met Gly Phe Ala Pro Tyr Gly Pro Tyr Trp Arg Glu Met Lys Lys Leu
115 120 125

Cys Ile Val His Leu Phe Ser Ala Gln Arg Val Arg Ser Phe Arg Pro
130 135 140

Ile Arg Glu Asn Glu Val Ala Lys Met Val Arg Lys Leu Ser Glu His
145 150 155 160

Glu Ala Ser Gly Thr Val Val Asn Leu Thr Glu Thr Leu Met Ser Phe
165 170 175

Thr Asn Ser Leu Ile Cys Arg Ile Ala Leu Gly Lys Ser Tyr Gly Cys
180 185 190

Glu Tyr Glu Glu Val Val Val Asp Glu Val Leu Gly Asn Arg Arg Ser
195 200 205

Arg Leu Gln Val Leu Leu Asn Glu Ala Gln Ala Leu Leu Ser Glu Phe
210 215 220

Phe Phe Ser Asp Tyr Phe Pro Pro Ile Gly Lys Trp Val Asp Arg Val
225 230 235 240

Thr Gly Ile Leu Ser Arg Leu Asp Lys Thr Phe Lys Glu Leu Asp Ala
245 250 255

Cys Tyr Glu Arg Ser Ser Tyr Asp His Met Asp Ser Ala Lys Ser Gly
260 265 270

Lys Lys Asp Asn Asp Asn Lys Glu Val Lys Asp Ile Ile Asp Ile Leu
275 280 285

Leu Gln Leu Leu Asp Asp Arg Ser Phe Thr Phe Asp Leu Thr Leu Asp
290 295 300

His Ile Lys Ala Val Leu Met Asn Ile Phe Ile Ala Gly Thr Asp Pro
305 310 315 320

Ser Ser Ala Thr Ile Val Trp Ala Met Asn Ala Leu Leu Lys Asn Pro
325 330 335

Asn Val Met Ser Lys Val Gln Gly Glu Val Arg Asn Leu Phe Gly Asp
340 345 350

```
Lys Asp Phe Ile Asn Glu Asp Asp Val Glu Ser Leu Pro Tyr Leu Lys
        355                 360                 365

Ala Val Val Lys Glu Thr Leu Arg Leu Phe Pro Pro Ser Pro Leu Leu
    370                 375                 380

Leu Pro Arg Val Thr Met Glu Thr Cys Asn Ile Glu Gly Tyr Glu Ile
385                 390                 395                 400

Gln Ala Lys Thr Ile Val His Val Asn Ala Trp Ala Ile Ala Arg Asp
                405                 410                 415

Pro Glu Asn Trp Glu Glu Pro Glu Lys Phe Phe Pro Glu Arg Phe Leu
            420                 425                 430

Glu Ser Ser Met Glu Leu Lys Gly Asn Asp Glu Phe Lys Val Ile Pro
        435                 440                 445

Phe Gly Ser Gly Arg Arg Met Cys Pro Ala Lys His Met Gly Ile Met
    450                 455                 460

Asn Val Glu Leu Ser Leu Ala Asn Leu Ile His Thr Phe Asp Trp Glu
465                 470                 475                 480

Val Ala Lys Gly Phe Asp Lys Glu Glu Met Leu Asp Thr Gln Met Lys
                485                 490                 495

Pro Gly Ile Thr Met His Lys Lys Ser Asp Leu Tyr Leu Val Ala Lys
            500                 505                 510

Lys Pro Thr Thr
        515
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1824 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 54..1616

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```
GGAAAATTAG CCTCACAAAA GCAAAGATCA AACAAACCAA GGACGAGAAC ACG ATG      56
                                                           Met
                                                            1

TTG CTT GAA CTT GCA CTT GGT TTA TTG GTT TTG GCT CTG TTT CTG CAC     104
Leu Leu Glu Leu Ala Leu Gly Leu Leu Val Leu Ala Leu Phe Leu His
  5                  10                  15
```

```
TTG CGT CCC ACA CCC ACT GCA AAA TCA AAA GCA CTT CGC CAT CTC CCA      152
Leu Arg Pro Thr Pro Thr Ala Lys Ser Lys Ala Leu Arg His Leu Pro
        20                  25                  30

AAC CCA CCA AGC CCA AAG CCT CGT CTT CCC TTC ATA GGA CAC CTT CAT      200
Asn Pro Pro Ser Pro Lys Pro Arg Leu Pro Phe Ile Gly His Leu His
    35                  40                  45

CTC TTA AAA GAC AAA CTT CTC CAC TAC GCA CTC ATC GAC CTC TCC AAA      248
Leu Leu Lys Asp Lys Leu Leu His Tyr Ala Leu Ile Asp Leu Ser Lys
50                  55                  60                  65

AAA CAT GGT CCC TTA TTC TCT CTC TAC TTT GGC TCC ATG CCA ACC GTT      296
Lys His Gly Pro Leu Phe Ser Leu Tyr Phe Gly Ser Met Pro Thr Val
                70                  75                  80

GTT GCC TCC ACA CCA GAA TTG TTC AAG CTC TTC CTC CAA ACG CAC GAG      344
Val Ala Ser Thr Pro Glu Leu Phe Lys Leu Phe Leu Gln Thr His Glu
            85                  90                  95

GCA ACT TCC TTC AAC ACA AGG TTC CAA ACC TCA GCC ATA AGA CGC CTC      392
Ala Thr Ser Phe Asn Thr Arg Phe Gln Thr Ser Ala Ile Arg Arg Leu
        100                 105                 110

ACC TAT GAT AGC TCA GTG GCC ATG GTT CCC TTC GGA CCT TAC TGG AAG      440
Thr Tyr Asp Ser Ser Val Ala Met Val Pro Phe Gly Pro Tyr Trp Lys
    115                 120                 125

TTC GTG AGG AAG CTC ATC ATG AAC GAC CTT CCC AAC GCC ACC ACT GTA      488
Phe Val Arg Lys Leu Ile Met Asn Asp Leu Pro Asn Ala Thr Thr Val
130                 135                 140                 145

AAC AAG TTG AGG CCT TTG AGG ACC CAA CAG ACC CGC AAG TTC CTT AGG      536
Asn Lys Leu Arg Pro Leu Arg Thr Gln Gln Thr Arg Lys Phe Leu Arg
                150                 155                 160

GTT ATG GCC CAA GGC GCA GAG GCA CAG AAG CCC CTT GAC TTG ACC GAG      584
Val Met Ala Gln Gly Ala Glu Ala Gln Lys Pro Leu Asp Leu Thr Glu
            165                 170                 175

GAG CTT CTG AAA TGG ACC AAC AGC ACC ATC TCC ATG ATG ATG CTC GGC      632
Glu Leu Leu Lys Trp Thr Asn Ser Thr Ile Ser Met Met Met Leu Gly
        180                 185                 190

GAG GCT GAG GAG ATC AGA GAC ATC GCT CGC GAG GTT CTT AAG ATC TTT      680
Glu Ala Glu Glu Ile Arg Asp Ile Ala Arg Glu Val Leu Lys Ile Phe
    195                 200                 205

GGC GAA TAC AGC CTC ACT GAC TTC ATC TGG CCA TTG AAG CAT CTC AAG      728
Gly Glu Tyr Ser Leu Thr Asp Phe Ile Trp Pro Leu Lys His Leu Lys
210                 215                 220                 225

GTT GGA AAG TAT GAG AAG AGG ATC GAC GAC ATC TTG AAC AAG TTC GAC      776
Val Gly Lys Tyr Glu Lys Arg Ile Asp Asp Ile Leu Asn Lys Phe Asp
                230                 235                 240

CCT GTC GTT GAA AGG GTC ATC AAG AAG CGC CGT GAG ATC GTG AGG AGG      824
Pro Val Val Glu Arg Val Ile Lys Lys Arg Arg Glu Ile Val Arg Arg
            245                 250                 255
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AGA AAG AAC GGA GAG GTT GTT GAG GGT GAG GTC AGC GGG GTT TTC CTT        872
Arg Lys Asn Gly Glu Val Val Glu Gly Glu Val Ser Gly Val Phe Leu
        260                 265                 270

GAC ACT TTC CTT GAA TTC GCT GAG GAT GAG ACC ATG GAG ATC AAA ATC        920
Asp Thr Leu Leu Glu Phe Ala Glu Asp Glu Thr Met Glu Ile Lys Ile
    275                 280                 285

ACC AAG GAC CAC ATC GAG GGT CTT GTT GTC GAC TTT TTC TCG GCA GGA        968
Thr Lys Asp His Ile Glu Gly Leu Val Val Asp Phe Phe Ser Ala Gly
290                 295                 300                 305

ACA GAC TCC ACA GCG GTG GCA ACA GAG TGG GCA TTG GCA GAA CTC ATC       1016
Thr Asp Ser Thr Ala Val Ala Thr Glu Trp Ala Leu Ala Glu Leu Ile
                310                 315                 320

AAC AAT CCT AAG GTG TTG GAA AAG GCT CGT GAG GAG GTC TAC AGT GTT       1064
Asn Asn Pro Lys Val Leu Glu Lys Ala Arg Glu Glu Val Tyr Ser Val
            325                 330                 335

GTG GGA AAG GAC AGA CTT GTG GAC GAA GTT GAC ACT CAA AAC CTT CCT       1112
Val Gly Lys Asp Arg Leu Val Asp Glu Val Asp Thr Gln Asn Leu Pro
        340                 345                 350

TAC ATT AGA GCA ATC GTG AAG GAG ACA TTC CGC ATG CAC CCG CCA CTC       1160
Tyr Ile Arg Ala Ile Val Lys Glu Thr Phe Arg Met His Pro Pro Leu
    355                 360                 365

CCA GTG GTC AAA AGA AAG TGC ACA GAA GAG TGT GAG ATT AAT GGA TAT       1208
Pro Val Val Lys Arg Lys Cys Thr Glu Glu Cys Glu Ile Asn Gly Tyr
370                 375                 380                 385

GTG ATC CCA GAG GGA GCA TTG ATT CTC TTC AAT GTA TGG CAA GTA GGA       1256
Val Ile Pro Glu Gly Ala Leu Ile Leu Phe Asn Val Trp Gln Val Gly
                390                 395                 400

AGA GAC CCC AAA TAC TGG GAC AGA CCA TCG GAG TTC CGT CCT GAG AGG       1304
Arg Asp Pro Lys Tyr Trp Asp Arg Pro Ser Glu Phe Arg Pro Glu Arg
            405                 410                 415

TTC CTA GAG ACA GGG GCT GAA GGG GAA GCA GGG CCT CTT GAT CTT AGG       1352
Phe Leu Glu Thr Gly Ala Glu Gly Glu Ala Gly Pro Leu Asp Leu Arg
        420                 425                 430

GGA CAA CAT TTT CAA CTT CTC CCA TTT GGG TCT GGG AGG AGA ATG TGC       1400
Gly Gln His Phe Gln Leu Leu Pro Phe Gly Ser Gly Arg Arg Met Cys
    435                 440                 445

CCT GGA GTC AAT CTG GCT ACT TCG GGA ATG GCA ACA CTT CTT GCA TCT       1448
Pro Gly Val Asn Leu Ala Thr Ser Gly Met Ala Thr Leu Leu Ala Ser
450                 455                 460                 465

CTT ATT CAG TGC TTC GAC TTG CAA GTG CTG GGT CCA CAA GGA CAG ATA       1496
Leu Ile Gln Cys Phe Asp Leu Gln Val Leu Gly Pro Gln Gly Gln Ile
                470                 475                 480

TTG AAG GGT GGT GAC GCC AAA GTT AGC ATG GAA GAG AGA GCC GGC CTC       1544
Leu Lys Gly Gly Asp Ala Lys Val Ser Met Glu Glu Arg Ala Gly Leu
            485                 490                 495
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ACT GTT CCA AGG GCA CAT AGT CTT GTC TGT GTT CCA CTT GCA AGG ATC      1592
Thr Val Pro Arg Ala His Ser Leu Val Cys Val Pro Leu Ala Arg Ile
        500                 505                 510

GGC GTT GCA TCT AAA CTC CTT TCT TAATTAAGAT CATCATCATA TATAATATTT     1646
Gly Val Ala Ser Lys Leu Leu Ser
    515                 520

ACTTTTTGTG TGTTGATAAT CATCATTTCA ATAAGGTCTC GTTCATCTAC TTTTTATGAA    1706

GTATATAAGC CCTTCCATGC ACATTGTATC ATCTCCCATT TGTCTTCGTT TGCTACCTAA    1766

GGCAATCTTT TTTTTTTTAG AATCACATCA TCCTACTATA AACTATCAAT CCTTATAT      1824
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 521 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Met Leu Leu Glu Leu Ala Leu Gly Leu Leu Val Leu Ala Leu Phe Leu
 1               5                  10                  15

His Leu Arg Pro Thr Pro Thr Ala Lys Ser Lys Ala Leu Arg His Leu
            20                  25                  30

Pro Asn Pro Pro Ser Pro Lys Pro Arg Leu Pro Phe Ile Gly His Leu
        35                  40                  45

His Leu Leu Lys Asp Lys Leu Leu His Tyr Ala Leu Ile Asp Leu Ser
    50                  55                  60

Lys Lys His Gly Pro Leu Phe Ser Leu Tyr Phe Gly Ser Met Pro Thr
65                  70                  75                  80

Val Val Ala Ser Thr Pro Glu Leu Phe Lys Leu Phe Leu Gln Thr His
                85                  90                  95

Glu Ala Thr Ser Phe Asn Thr Arg Phe Gln Thr Ser Ala Ile Arg Arg
            100                 105                 110

Leu Thr Tyr Asp Ser Ser Val Ala Met Val Pro Phe Gly Pro Tyr Trp
        115                 120                 125

Lys Phe Val Arg Lys Leu Ile Met Asn Asp Leu Pro Asn Ala Thr Thr
    130                 135                 140

Val Asn Lys Leu Arg Pro Leu Arg Thr Gln Gln Thr Arg Lys Phe Leu
145                 150                 155                 160

Arg Val Met Ala Gln Gly Ala Glu Ala Gln Lys Pro Leu Asp Leu Thr
                165                 170                 175

Glu Glu Leu Leu Lys Trp Thr Asn Ser Thr Ile Ser Met Met Met Leu
```

180 185 190

Gly Glu Ala Glu Glu Ile Arg Asp Ile Ala Arg Glu Val Leu Lys Ile
195 200 205

Phe Gly Glu Tyr Ser Leu Thr Asp Phe Ile Trp Pro Leu Lys His Leu
210 215 220

Lys Val Gly Lys Tyr Glu Lys Arg Ile Asp Asp Ile Leu Asn Lys Phe
225 230 235 240

Asp Pro Val Val Glu Arg Val Ile Lys Lys Arg Arg Glu Ile Val Arg
245 250 255

Arg Arg Lys Asn Gly Glu Val Val Glu Gly Glu Val Ser Gly Val Phe
260 265 270

Leu Asp Thr Leu Leu Glu Phe Ala Glu Asp Glu Thr Met Glu Ile Lys
275 280 285

Ile Thr Lys Asp His Ile Glu Gly Leu Val Val Asp Phe Phe Ser Ala
290 295 300

Gly Thr Asp Ser Thr Ala Val Ala Thr Glu Trp Ala Leu Ala Glu Leu
305 310 315 320

Ile Asn Asn Pro Lys Val Leu Glu Lys Ala Arg Glu Glu Val Tyr Ser
325 330 335

Val Val Gly Lys Asp Arg Leu Val Asp Glu Val Asp Thr Gln Asn Leu
340 345 350

Pro Tyr Ile Arg Ala Ile Val Lys Glu Thr Phe Arg Met His Pro Pro
355 360 365

Leu Pro Val Val Lys Arg Lys Cys Thr Glu Glu Cys Glu Ile Asn Gly
370 375 380

Tyr Val Ile Pro Glu Gly Ala Leu Ile Leu Phe Asn Val Trp Gln Val
385 390 395 400

Gly Arg Asp Pro Lys Tyr Trp Asp Arg Pro Ser Glu Phe Arg Pro Glu
405 410 415

Arg Phe Leu Glu Thr Gly Ala Glu Gly Glu Ala Gly Pro Leu Asp Leu
420 425 430

Arg Gly Gln His Phe Gln Leu Leu Pro Phe Gly Ser Gly Arg Arg Met
435 440 445

Cys Pro Gly Val Asn Leu Ala Thr Ser Gly Met Ala Thr Leu Leu Ala
450 455 460

Ser Leu Ile Gln Cys Phe Asp Leu Gln Val Leu Gly Pro Gln Gly Gln
465 470 475 480

Ile Leu Lys Gly Gly Asp Ala Lys Val Ser Met Glu Glu Arg Ala Gly
485 490 495

Leu Thr Val Pro Arg Ala His Ser Leu Val Cys Val Pro Leu Ala Arg

500 505 510

Ile Gly Val Ala Ser Lys Leu Leu Ser
515 520

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1831 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 20..1747

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
CAACACTCGC AGTACCGCC ATG AGT GTC GAC ACT TCC TCC ACC CTC TCC ACC      52
                      Met Ser Val Asp Thr Ser Ser Thr Leu Ser Thr
                        1               5                  10

GTC ACC GAT GCC AAT CTT CAC TCC AGA TTT CAT TCT CGT CTT GTT CCA      100
Val Thr Asp Ala Asn Leu His Ser Arg Phe His Ser Arg Leu Val Pro
            15                  20                  25

TTC ACT CAT CAT TTC TCA CTT TCT CAA CCC AAA CGG ATT TCT TCA ATC      148
Phe Thr His His Phe Ser Leu Ser Gln Pro Lys Arg Ile Ser Ser Ile
        30                  35                  40

AGA TGC CAA TCA ATT AAT ACC GAT AAG AAG AAA TCA AGT AGA AAT CTG      196
Arg Cys Gln Ser Ile Asn Thr Asp Lys Lys Lys Ser Ser Arg Asn Leu
    45                  50                  55

CTG GGC AAT GCA AGT AAC CTC CTC ACG GAC TTA TTA AGT GGT GGA AGT      244
Leu Gly Asn Ala Ser Asn Leu Leu Thr Asp Leu Leu Ser Gly Gly Ser
60                  65                  70                  75

ATA GGG TCT ATG CCC ATA GCT GAA GGT GCA GTC TCA GAT CTG CTT GGT      292
Ile Gly Ser Met Pro Ile Ala Glu Gly Ala Val Ser Asp Leu Leu Gly
                80                  85                  90

CGA CCT CTC TTT TTC TCA CTG TAT GAT TGG TTC TTG GAG CAT GGT GCG      340
Arg Pro Leu Phe Phe Ser Leu Tyr Asp Trp Phe Leu Glu His Gly Ala
            95                  100                 105

GTG TAT AAA CTT GCC TTT GGA CCA AAA GCA TTT GTT GTT GTA TCA GAT      388
Val Tyr Lys Leu Ala Phe Gly Pro Lys Ala Phe Val Val Val Ser Asp
        110                 115                 120

CCC ATA GTT GCT AGA CAT ATT CTG CGA GAA AAT GCA TTT TCT TAT GAC      436
Pro Ile Val Ala Arg His Ile Leu Arg Glu Asn Ala Phe Ser Tyr Asp
    125                 130                 135

AAG GGA GTA CTT GCT GAT ATC CTT GAA CCA ATA ATG GGC AAA GGA CTC      484
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Lys Gly Val Leu Ala Asp Ile Leu Glu Pro Ile Met Gly Lys Gly Leu
140                 145                 150                 155

ATA CCA GCA GAC CTT GAT ACT TGG AAG CAA AGG AGA AGA GTC ATT GCT      532
Ile Pro Ala Asp Leu Asp Thr Trp Lys Gln Arg Arg Arg Val Ile Ala
                160                 165                 170

CCG GCT TTC CAT AAC TCA TAC TTG GAA GCT ATG GTT AAA ATA TTC ACA      580
Pro Ala Phe His Asn Ser Tyr Leu Glu Ala Met Val Lys Ile Phe Thr
            175                 180                 185

ACT TGT TCA GAA AGA ACA ATA TTG AAG TTT AAT AAG CTT CTT GAA GGA      628
Thr Cys Ser Glu Arg Thr Ile Leu Lys Phe Asn Lys Leu Leu Glu Gly
        190                 195                 200

GAG GGT TAT GAT GGA CCT GAC TCA ATT GAA TTG GAT CTT GAG GCA GAG      676
Glu Gly Tyr Asp Gly Pro Asp Ser Ile Glu Leu Asp Leu Glu Ala Glu
    205                 210                 215

TTT TCT AGT TTG GCT CTT GAT ATT ATT GGG CTT GGT GTG TTC AAC TAT      724
Phe Ser Ser Leu Ala Leu Asp Ile Ile Gly Leu Gly Val Phe Asn Tyr
220                 225                 230                 235

GAC TTT GGT TCT GTC ACC AAA GAA TCT CCA GTT ATT AAG GCA GTC TAT      772
Asp Phe Gly Ser Val Thr Lys Glu Ser Pro Val Ile Lys Ala Val Tyr
                240                 245                 250

GGC ACT CTT TTT GAA GCT GAA CAC AGA TCC ACT TTC TAC ATT CCA TAT      820
Gly Thr Leu Phe Glu Ala Glu His Arg Ser Thr Phe Tyr Ile Pro Tyr
            255                 260                 265

TGG AAA ATT CCA TTG GCA AGG TGG ATA GTC CCA AGG CAA AGA AAG TTT      868
Trp Lys Ile Pro Leu Ala Arg Trp Ile Val Pro Arg Gln Arg Lys Phe
        270                 275                 280

CAG GAT GAC CTA AAG GTC ATC AAT ACT TGT CTT GAT GGA CTT ATC AGA      916
Gln Asp Asp Leu Lys Val Ile Asn Thr Cys Leu Asp Gly Leu Ile Arg
    285                 290                 295

AAT GCA AAA GAG AGC AGA CAG GAA ACA GAT GTT GAG AAA TTG CAG CAG      964
Asn Ala Lys Glu Ser Arg Gln Glu Thr Asp Val Glu Lys Leu Gln Gln
300                 305                 310                 315

AGG GAT TAC TTA AAT TTG AAG GAT GCA AGT CTT CTG CGT TTC CTG GTT     1012
Arg Asp Tyr Leu Asn Leu Lys Asp Ala Ser Leu Leu Arg Phe Leu Val
                320                 325                 330

GAT ATG CGG GGA GCT GAT GTT GAT GAT CGT CAG TTG AGG GAT GAT TTA     1060
Asp Met Arg Gly Ala Asp Val Asp Asp Arg Gln Leu Arg Asp Asp Leu
            335                 340                 345

ATG ACA ATG CTT ATT GCC GGT CAT GAA ACA ACG GCT GCA GTT CTT ACT     1108
Met Thr Met Leu Ile Ala Gly His Glu Thr Thr Ala Ala Val Leu Thr
        350                 355                 360

TGG GCA GTT TTC CTC CTA GCT CAA AAT CCT AGC AAA ATG AAG AAG GCT     1156
Trp Ala Val Phe Leu Leu Ala Gln Asn Pro Ser Lys Met Lys Lys Ala
    365                 370                 375
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CAA GCA GAG GTA GAT TTG GTG CTG GGT ACG GGG AGG CCA ACT TTT GAA      1204
Gln Ala Glu Val Asp Leu Val Leu Gly Thr Gly Arg Pro Thr Phe Glu
380                 385                 390                 395

TCA CTT AAG GAA TTG CAG TAC ATT AGA TTG ATT GTT GTG GAG GCT CTT      1252
Ser Leu Lys Glu Leu Gln Tyr Ile Arg Leu Ile Val Val Glu Ala Leu
                400                 405                 410

CGT TTA TAC CCC CAA CCA CCT TTG CTG ATT AGA CGT TCA CTC AAA TCT      1300
Arg Leu Tyr Pro Gln Pro Pro Leu Leu Ile Arg Arg Ser Leu Lys Ser
            415                 420                 425

GAT GTT TTA CCA GGT GGG CAC AAA GGT GAA AAA GAT GGT TAT GCA ATT      1348
Asp Val Leu Pro Gly Gly His Lys Gly Glu Lys Asp Gly Tyr Ala Ile
        430                 435                 440

CCT GCT GGG ACT GAT GTC TTC ATT TCT GTA TAT AAT CTC CAT AGA TCT      1396
Pro Ala Gly Thr Asp Val Phe Ile Ser Val Tyr Asn Leu His Arg Ser
    445                 450                 455

CCA TAT TTT TGG GAC CGC CCT GAT GAC TTC GAA CCA GAG AGA TTT CTT      1444
Pro Tyr Phe Trp Asp Arg Pro Asp Asp Phe Glu Pro Glu Arg Phe Leu
460                 465                 470                 475

GTG CAA AAC AAG AAT GAA GAA ATT GAA GGA TGG GCT GGT CTT GAT CCA      1492
Val Gln Asn Lys Asn Glu Glu Ile Glu Gly Trp Ala Gly Leu Asp Pro
                480                 485                 490

TCT CGA AGT CCC GGA GCC TTG TAT CCG AAC GAG GTT ATA TCG GAT TTT      1540
Ser Arg Ser Pro Gly Ala Leu Tyr Pro Asn Glu Val Ile Ser Asp Phe
            495                 500                 505

GCA TTC TTA CCT TTT GGT GGC GGA CCA CGA AAA TGT GTT GGG GAC CAA      1588
Ala Phe Leu Pro Phe Gly Gly Gly Pro Arg Lys Cys Val Gly Asp Gln
        510                 515                 520

TTT GCT CTG ATG GAG TCC ACT GTA GCG TTG ACT ATG CTG CTC CAG AAT      1636
Phe Ala Leu Met Glu Ser Thr Val Ala Leu Thr Met Leu Leu Gln Asn
    525                 530                 535

TTT GAC GTG GAA CTA AAA GGG ACC CCT GAA TCG GTG GAA CTA GTT ACT      1684
Phe Asp Val Glu Leu Lys Gly Thr Pro Glu Ser Val Glu Leu Val Thr
540                 545                 550                 555

GGG GCA ACT ATT CAT ACC AAA AAT GGA ATG TGG TGC AGA TTG AAG AAG      1732
Gly Ala Thr Ile His Thr Lys Asn Gly Met Trp Cys Arg Leu Lys Lys
                560                 565                 570

AGA TCT AAT TTA CGT TGACATATGT ACTGTGGCCA TTTTTCTTAT ACAGAATAAT      1787
Arg Ser Asn Leu Arg
            575

GTATATTATT ATTCTTTGAG AATAATATGA ATAAATTCCT AGAC                     1831
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 576 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Ser Val Asp Thr Ser Ser Thr Leu Ser Thr Val Thr Asp Ala Asn
1 5 10 15

Leu His Ser Arg Phe His Ser Arg Leu Val Pro Phe Thr His His Phe
20 25 30

Ser Leu Ser Gln Pro Lys Arg Ile Ser Ser Ile Arg Cys Gln Ser Ile
35 40 45

Asn Thr Asp Lys Lys Lys Ser Ser Arg Asn Leu Leu Gly Asn Ala Ser
50 55 60

Asn Leu Leu Thr Asp Leu Leu Ser Gly Gly Ser Ile Gly Ser Met Pro
65 70 75 80

Ile Ala Glu Gly Ala Val Ser Asp Leu Leu Gly Arg Pro Leu Phe Phe
85 90 95

Ser Leu Tyr Asp Trp Phe Leu Glu His Gly Ala Val Tyr Lys Leu Ala
100 105 110

Phe Gly Pro Lys Ala Phe Val Val Val Ser Asp Pro Ile Val Ala Arg
115 120 125

His Ile Leu Arg Glu Asn Ala Phe Ser Tyr Asp Lys Gly Val Leu Ala
130 135 140

Asp Ile Leu Glu Pro Ile Met Gly Lys Gly Leu Ile Pro Ala Asp Leu
145 150 155 160

Asp Thr Trp Lys Gln Arg Arg Arg Val Ile Ala Pro Ala Phe His Asn
165 170 175

Ser Tyr Leu Glu Ala Met Val Lys Ile Phe Thr Thr Cys Ser Glu Arg
180 185 190

Thr Ile Leu Lys Phe Asn Lys Leu Leu Glu Gly Glu Gly Tyr Asp Gly
195 200 205

Pro Asp Ser Ile Glu Leu Asp Leu Glu Ala Glu Phe Ser Ser Leu Ala
210 215 220

Leu Asp Ile Ile Gly Leu Gly Val Phe Asn Tyr Asp Phe Gly Ser Val
225 230 235 240

Thr Lys Glu Ser Pro Val Ile Lys Ala Val Tyr Gly Thr Leu Phe Glu
245 250 255

Ala Glu His Arg Ser Thr Phe Tyr Ile Pro Tyr Trp Lys Ile Pro Leu
260 265 270

Ala Arg Trp Ile Val Pro Arg Gln Arg Lys Phe Gln Asp Asp Leu Lys
275 280 285

Val Ile Asn Thr Cys Leu Asp Gly Leu Ile Arg Asn Ala Lys Glu Ser
290 295 300

Arg Gln Glu Thr Asp Val Glu Lys Leu Gln Gln Arg Asp Tyr Leu Asn
305 310 315 320

Leu Lys Asp Ala Ser Leu Leu Arg Phe Leu Val Asp Met Arg Gly Ala
325 330 335

Asp Val Asp Asp Arg Gln Leu Arg Asp Asp Leu Met Thr Met Leu Ile
340 345 350

Ala Gly His Glu Thr Thr Ala Ala Val Leu Thr Trp Ala Val Phe Leu
355 360 365

Leu Ala Gln Asn Pro Ser Lys Met Lys Lys Ala Gln Ala Glu Val Asp
370 375 380

Leu Val Leu Gly Thr Gly Arg Pro Thr Phe Glu Ser Leu Lys Glu Leu
385 390 395 400

Gln Tyr Ile Arg Leu Ile Val Val Glu Ala Leu Arg Leu Tyr Pro Gln
405 410 415

Pro Pro Leu Leu Ile Arg Arg Ser Leu Lys Ser Asp Val Leu Pro Gly
420 425 430

Gly His Lys Gly Glu Lys Asp Gly Tyr Ala Ile Pro Ala Gly Thr Asp
435 440 445

Val Phe Ile Ser Val Tyr Asn Leu His Arg Ser Pro Tyr Phe Trp Asp
450 455 460

Arg Pro Asp Asp Phe Glu Pro Glu Arg Phe Leu Val Gln Asn Lys Asn
465 470 475 480

Glu Glu Ile Glu Gly Trp Ala Gly Leu Asp Pro Ser Arg Ser Pro Gly
485 490 495

Ala Leu Tyr Pro Asn Glu Val Ile Ser Asp Phe Ala Phe Leu Pro Phe
500 505 510

Gly Gly Gly Pro Arg Lys Cys Val Gly Asp Gln Phe Ala Leu Met Glu
515 520 525

Ser Thr Val Ala Leu Thr Met Leu Leu Gln Asn Phe Asp Val Glu Leu
530 535 540

Lys Gly Thr Pro Glu Ser Val Glu Leu Val Thr Gly Ala Thr Ile His
545 550 555 560

Thr Lys Asn Gly Met Trp Cys Arg Leu Lys Lys Arg Ser Asn Leu Arg
565 570 575

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1704 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 38..1564

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
CAGGCTCCAC AAAACATCTC ATCATTCACC CAACAAA ATG GCG CTG CTT CTG ATA        55
                                          Met Ala Leu Leu Leu Ile
                                            1               5

ATT CCC ATC TCA CTG GTC ACC CTC TGG CTC GGT TAC ACC CTA TAC CAG       103
Ile Pro Ile Ser Leu Val Thr Leu Trp Leu Gly Tyr Thr Leu Tyr Gln
            10                  15                  20

CGA TTA AGA TTC AAG CTC CCT CCG GGT CCA CGG CCC TGG CCG GTA GTC       151
Arg Leu Arg Phe Lys Leu Pro Pro Gly Pro Arg Pro Trp Pro Val Val
        25                  30                  35

GGT AAC CTC TAC GAC ATA AAA CCC GTC CGC TTC CGG TGC TTC GCG GAG       199
Gly Asn Leu Tyr Asp Ile Lys Pro Val Arg Phe Arg Cys Phe Ala Glu
    40                  45                  50

TGG GCG CAG TCT TAC GGC CCC ATA ATA TCG GTT TGG TTC GGT TCG ACC       247
Trp Ala Gln Ser Tyr Gly Pro Ile Ile Ser Val Trp Phe Gly Ser Thr
55                  60                  65                  70

CTA AAC GTC ATC GTT TCG AAC TCG GAG CTG GCG AAG GAG GTG CTG AAG       295
Leu Asn Val Ile Val Ser Asn Ser Glu Leu Ala Lys Glu Val Leu Lys
                75                  80                  85

GAG CAC GAT CAG CTG CTG GCG GAC CGC CAC CGG AGC CGG TCG GCG GCG       343
Glu His Asp Gln Leu Leu Ala Asp Arg His Arg Ser Arg Ser Ala Ala
            90                  95                  100

AAG TTC AGC CGC GAC GGG AAG GAT CTA ATT TGG GCC GAT TAT GGG CCG       391
Lys Phe Ser Arg Asp Gly Lys Asp Leu Ile Trp Ala Asp Tyr Gly Pro
        105                 110                 115

CAC TAC GTG AAG GTG AGG AAG GTT TGC ACG CTC GAG CTT TTC TCG CCG       439
His Tyr Val Lys Val Arg Lys Val Cys Thr Leu Glu Leu Phe Ser Pro
    120                 125                 130

AAG CGC CTC GAG GCC CTG AGG CCC ATT AGG GAG GAC GAG GTC ACC TCC       487
Lys Arg Leu Glu Ala Leu Arg Pro Ile Arg Glu Asp Glu Val Thr Ser
135                 140                 145                 150

ATG GTT GAC TCC GTT TAC AAT CAC TGC ACC AGC ACT GAA AAT TTG GGG       535
Met Val Asp Ser Val Tyr Asn His Cys Thr Ser Thr Glu Asn Leu Gly
                155                 160                 165

AAA GGA ATA TTG TTG AGG AAG CAC TTG GGG GTT GTG GCA TTC AAC AAC       583
Lys Gly Ile Leu Leu Arg Lys His Leu Gly Val Val Ala Phe Asn Asn
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                170                 175                 180

ATA ACC AGG TTG GCA TTT GGG AAA AGA TTT GTG AAC TCA GAA GGT GTG      631
Ile Thr Arg Leu Ala Phe Gly Lys Arg Phe Val Asn Ser Glu Gly Val
        185                 190                 195

ATG GAT GAG CAA GGA GTA GAA TTC AAG GCC ATT GTG GAA AAT GGG TTA      679
Met Asp Glu Gln Gly Val Glu Phe Lys Ala Ile Val Glu Asn Gly Leu
    200                 205                 210

AAG CTA GGA GCA TCT CTA GCC ATG GCA GAA CAC ATC CCT TGG CTT CGC      727
Lys Leu Gly Ala Ser Leu Ala Met Ala Glu His Ile Pro Trp Leu Arg
215                 220                 225                 230

TGG ATG TTC CCA CTG GAA GAA GGA GCT TTT GCC AAG CAT GGA GCC CGC      775
Trp Met Phe Pro Leu Glu Glu Gly Ala Phe Ala Lys His Gly Ala Arg
                235                 240                 245

CGC GAC CGA CTC ACC AGA GCC ATC ATG GCA GAG CAC ACT GAA GCA CGC      823
Arg Asp Arg Leu Thr Arg Ala Ile Met Ala Glu His Thr Glu Ala Arg
            250                 255                 260

AAG AAA TCT GGT GGT GCC AAG CAA CAT TTT GTT GAT GCC CTC CTC ACA      871
Lys Lys Ser Gly Gly Ala Lys Gln His Phe Val Asp Ala Leu Leu Thr
        265                 270                 275

TTG CAA GAC AAA TAT GAC CTT AGT GAA GAC ACC ATC ATT GGT CTC CTT      919
Leu Gln Asp Lys Tyr Asp Leu Ser Glu Asp Thr Ile Ile Gly Leu Leu
    280                 285                 290

TGG GAT ATG ATC ACA GCA GGG ATG GAC ACA ACT GCA ATT TCA GTT GAG      967
Trp Asp Met Ile Thr Ala Gly Met Asp Thr Thr Ala Ile Ser Val Glu
295                 300                 305                 310

TGG GCC ATG GCT GAG TTG ATA AGA AAC CCA AGG GTG CAA CAA AAG GTC     1015
Trp Ala Met Ala Glu Leu Ile Arg Asn Pro Arg Val Gln Gln Lys Val
                315                 320                 325

CAA GAG GAG CTA GAC AGG GTA ATT GGG CTT GAA AGG GTG ATG ACT GAA     1063
Gln Glu Glu Leu Asp Arg Val Ile Gly Leu Glu Arg Val Met Thr Glu
            330                 335                 340

GCA GAC TTC TCA AAT CTC CCT TAC CTA CAA TGT GTG ACC AAA GAA GCA     1111
Ala Asp Phe Ser Asn Leu Pro Tyr Leu Gln Cys Val Thr Lys Glu Ala
        345                 350                 355

ATG AGG CTT CAC CCA CCA ACC CCA CTA ATG CTC CCA CAC CGT GCC AAT     1159
Met Arg Leu His Pro Pro Thr Pro Leu Met Leu Pro His Arg Ala Asn
    360                 365                 370

GCC AAT GTC AAA GTT GGA GGC TAT GAC ATT CCC AAA GGG TCC AAT GTG     1207
Ala Asn Val Lys Val Gly Gly Tyr Asp Ile Pro Lys Gly Ser Asn Val
375                 380                 385                 390

CAT GTG AAT GTG TGG GCG GTG GCC CGC GAC CCG GCC GTG TGG AAG GAT     1255
His Val Asn Val Trp Ala Val Ala Arg Asp Pro Ala Val Trp Lys Asp
                395                 400                 405

CCA TTG GAG TTC CGA CCC GAA AGG TTC CTT GAG GAG GAT GTA GAC ATG     1303
Pro Leu Glu Phe Arg Pro Glu Arg Phe Leu Glu Glu Asp Val Asp Met
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            410                 415                 420

AAG GGC CAT GAC TTT AGG CTA CTT CCA TTC GGG TCG GGT CGA CGA GTA      1351
Lys Gly His Asp Phe Arg Leu Leu Pro Phe Gly Ser Gly Arg Arg Val
        425                 430                 435

TGC CCG GGT GCC CAA CTT GGT ATC AAC TTG GCA GCA TCC ATG TTG GGC      1399
Cys Pro Gly Ala Gln Leu Gly Ile Asn Leu Ala Ala Ser Met Leu Gly
    440                 445                 450

CAC CTC TTG CAC CAT TTC TGT TGG ACC CCA CCT GAA GGA ATG AAG CCT      1447
His Leu Leu His His Phe Cys Trp Thr Pro Pro Glu Gly Met Lys Pro
455                 460                 465                 470

GAG GAA ATT GAC ATG GGA GAG AAT CCA GGG CTA GTC ACA TAC ATG AGG      1495
Glu Glu Ile Asp Met Gly Glu Asn Pro Gly Leu Val Thr Tyr Met Arg
                475                 480                 485

ACT CCA ATA CAA GCT GTG GTT TCT CCT AGG CTC CCC TCA CAT TTA TAC      1543
Thr Pro Ile Gln Ala Val Val Ser Pro Arg Leu Pro Ser His Leu Tyr
            490                 495                 500

AAA CGT GTG CCT GCT GAG ATC TAATCTTTCT TTTCTTTCCC TTGGACTACT         1594
Lys Arg Val Pro Ala Glu Ile
        505

CTTTGTTGCA TTAAGAAAAA TGCCTTGTGG CACTACTTTT ATCTTTGTGT TTATGTAACT    1654

ACATATGAAA TCACAATTTA AGGAACTAAG GAAAAACTCA TTGCGAGGGT              1704
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 509 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Met Ala Leu Leu Leu Ile Ile Pro Ile Ser Leu Val Thr Leu Trp Leu
  1               5                  10                  15

Gly Tyr Thr Leu Tyr Gln Arg Leu Arg Phe Lys Leu Pro Pro Gly Pro
            20                  25                  30

Arg Pro Trp Pro Val Val Gly Asn Leu Tyr Asp Ile Lys Pro Val Arg
        35                  40                  45

Phe Arg Cys Phe Ala Glu Trp Ala Gln Ser Tyr Gly Pro Ile Ile Ser
    50                  55                  60

Val Trp Phe Gly Ser Thr Leu Asn Val Ile Val Ser Asn Ser Glu Leu
65                  70                  75                  80

Ala Lys Glu Val Leu Lys Glu His Asp Gln Leu Leu Ala Asp Arg His
                85                  90                  95
```

Arg Ser Arg Ser Ala Ala Lys Phe Ser Arg Asp Gly Lys Asp Leu Ile
100 105 110

Trp Ala Asp Tyr Gly Pro His Tyr Val Lys Val Arg Lys Val Cys Thr
115 120 125

Leu Glu Leu Phe Ser Pro Lys Arg Leu Glu Ala Leu Arg Pro Ile Arg
130 135 140

Glu Asp Glu Val Thr Ser Met Val Asp Ser Val Tyr Asn His Cys Thr
145 150 155 160

Ser Thr Glu Asn Leu Gly Lys Gly Ile Leu Leu Arg Lys His Leu Gly
165 170 175

Val Val Ala Phe Asn Asn Ile Thr Arg Leu Ala Phe Gly Lys Arg Phe
180 185 190

Val Asn Ser Glu Gly Val Met Asp Glu Gln Gly Val Glu Phe Lys Ala
195 200 205

Ile Val Glu Asn Gly Leu Lys Leu Gly Ala Ser Leu Ala Met Ala Glu
210 215 220

His Ile Pro Trp Leu Arg Trp Met Phe Pro Leu Glu Glu Gly Ala Phe
225 230 235 240

Ala Lys His Gly Ala Arg Arg Asp Arg Leu Thr Arg Ala Ile Met Ala
245 250 255

Glu His Thr Glu Ala Arg Lys Lys Ser Gly Gly Ala Lys Gln His Phe
260 265 270

Val Asp Ala Leu Leu Thr Leu Gln Asp Lys Tyr Asp Leu Ser Glu Asp
275 280 285

Thr Ile Ile Gly Leu Leu Trp Asp Met Ile Thr Ala Gly Met Asp Thr
290 295 300

Thr Ala Ile Ser Val Glu Trp Ala Met Ala Glu Leu Ile Arg Asn Pro
305 310 315 320

Arg Val Gln Gln Lys Val Gln Glu Glu Leu Asp Arg Val Ile Gly Leu
325 330 335

Glu Arg Val Met Thr Glu Ala Asp Phe Ser Asn Leu Pro Tyr Leu Gln
340 345 350

Cys Val Thr Lys Glu Ala Met Arg Leu His Pro Pro Thr Pro Leu Met
355 360 365

Leu Pro His Arg Ala Asn Ala Asn Val Lys Val Gly Gly Tyr Asp Ile
370 375 380

Pro Lys Gly Ser Asn Val His Val Asn Val Trp Ala Val Ala Arg Asp
385 390 395 400

Pro Ala Val Trp Lys Asp Pro Leu Glu Phe Arg Pro Glu Arg Phe Leu
405 410 415

Glu Glu Asp Val Asp Met Lys Gly His Asp Phe Arg Leu Leu Pro Phe
420 425 430

Gly Ser Gly Arg Arg Val Cys Pro Gly Ala Gln Leu Gly Ile Asn Leu
435 440 445

Ala Ala Ser Met Leu Gly His Leu Leu His His Phe Cys Trp Thr Pro
450 455 460

Pro Glu Gly Met Lys Pro Glu Glu Ile Asp Met Gly Glu Asn Pro Gly
465 470 475 480

Leu Val Thr Tyr Met Arg Thr Pro Ile Gln Ala Val Val Ser Pro Arg
485 490 495

Leu Pro Ser His Leu Tyr Lys Arg Val Pro Ala Glu Ile
500 505

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGTCTAACTC CTTCCTTTTC 20

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Phe Leu Pro Phe Gly Xaa Gly Xaa Arg Xaa Cys Xaa Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Phe Xaa Xaa Gly Xaa Xaa Xaa Cys Xaa Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Xaa Cys Xaa Gly
1

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Pro Glu Glu Phe Xaa Pro Glu Arg Phe
1 5

INTERNATIONAL SEARCH REPORT

Ir [redacted]al Application No
PCT/US 98/20807

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/53 C12N15/82 C12N9/02 C12N5/00 A01H5/00
A01H5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SUZUKI G ET AL: "Induction of a novel cytochrome P450 (CYP93 family) b methyl jasmonate in soybean suspension-cultured cells." FEBS LETTERS, (1996 MAR 25) 383 (1-2) 83-6. JOURNAL CODE: EUH. ISSN: 0014-5793., XP002046657 Netherlands see the whole document ---	1-47
A	DATABASE WPI Section Ch, Week 9745 Derwent Publications Ltd., London, GB; Class C12,Page 10, AN 97-484100 XP002100401 & JP 09 224671 A (MITSUI GYOSAI SHOKUBUTSU BIO KENKYUSHO), 2 September 1997 see abstract ---	1-47

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 April 1999	03/05/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 03561 A (DU PONT) 21 March 1991 cited in the application see the whole document	1-47
A	FRANK M. ET AL.: "Cloning of wound-induced cytochrome P450 monooxygenases expressed in pea" PLANT PHYSIOLOGY, vol. 110, 1996, pages 1035-1046, XP002100394 see the whole document	1-47
A	SHIOTA N. ET AL.: "Herbicide-resistant tobacco plants expressing the fused enzyme between rat cytochrome P4501A1 (CYP1A1) and yeast NADPH-cytochrome P450 oxidoreductase" PLANT PHYSIOLOGY, vol. 106, 1994, pages 17-23, XP002100395 cited in the application see the whole document	1-47
A	PIERREL M. ET AL.: "Catalytic properties of the plant cytochrome P450 CYP73 expressed in yeast" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 224, no. 3, 1994, pages 835-844, XP002100396 cited in the application see the whole document	1-47
A	KOCHS G. ET AL.: "Further characterization of cytochrome P450 in phytoalexin synthesis in soybean: cytochrome P450 cinnamate 4-hydroxylase and 3,9-dihydroxypterocarpan 6a-hydroxylase" ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS, vol. 293, no. 1, 1992, pages 187-194, XP002100397 see the whole document	1-47
P,X	SIMINSZKY B. ET AL.: "AC AF022157" EMBL DATABASE,8 January 1998, XP002100398 see the whole document	1-47
T	SCHOPFER C R ET AL: "Identification of elicitor-induced cytochrome P450s of soybean Glycine max L.) using differential display of mRNA." MOLECULAR AND GENERAL GENETICS, (1998 MAY) 258 (4) 315-22. JOURNAL CODE: NGP. ISSN: 0026-8925., XP002100399 GERMANY: Germany, Federal Republic of see the whole document	1-47
	-/--	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

In [redacted] Application No
PCT/US 98/20807

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
T	SIMINSZKY B ET AL: "Expression of a soybean cytochrome P450 monooxygenase cDNA in yeast and tobacco enhances the metabolism of phenylurea herbicides." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 FEB 16) 96 (4) 1750-5. JOURNAL CODE: PV3. ISSN: 0027-8424., XP002100400 United States see the whole document	1-47

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/ 20807

Box I Observations where certain claims w re found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant s protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-3,7-16 partially; 4-6,17-47 completely
An isolated DNA molecule comprising a sequence consisting of SEQ ID NO:1, coding for an enzyme of SEQ ID NO:2, DNA sequences at least 90 % identical thereto and encoding a cytochrome P450 enzyme, and variants thereof. Encoded peptides, P450 enzymes, DNA constructs therewith, plant cells and transgenic plants comprising said constructs. A method of making a transgenic plant cell having an increased ability to metabolize phenylurea compounds compared to an untransformed cell, by transformation with said construct, and plants having increased resistance to phenylurea herbicides compared to wild-type plants of the same species, progeny and seed thereof. A crop comprising said plants. A method of using a phenylurea herbicide as a post-emergence herbicide, comprising planting said crop and applying a phenylurea herbicide thereto.

2. Claims : 1-3,7-16 partially
An isolated DNA molecule comprising a sequence consisting of SEQ NO:3, coding for an enzyme of SEQ IS NO: 4, DNA sequences at least 90% identical thereto and encoding a cytochrome P450 enzyme, and variants thereof. Encoded peptides, P450, DNA constructs therewith, plant cells and transgenic plants comprising said constructs.
3. Claims : 1-3,7-16 partially
idem for SED ID NOs: 5,6
4. Claims : 1-3,7-16 partially
idem for SED ID NOs: 7,8
5. Claims : 1-3,7-16 partially
idem for SED ID NOs: 9,10
6. Claims : 1-3,7-16 partially
idem for SED ID NOs: 11,12
7. Claims : 1-3,7-16 partially
idem for SED ID NOs: 13,14
8. Claims : 1-3,7-16 partially
idem for SED ID NOs: 15,16
9. Claims : 1-3,7-16 partially
idem for SED ID NOs: 17,18

INTERNATIONAL SEARCH REPORT

...mation on patent family members

...ational Application No
PCT/US 98/20807

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9103561 A	21-03-1991	US 5212296 A	18-05-1993
		AT 133201 T	15-02-1996
		AU 648036 B	14-04-1994
		AU 6272990 A	08-04-1991
		CA 2065439 A	12-03-1991
		DE 69024979 D	29-02-1996
		DE 69024979 T	17-10-1996
		DK 554240 T	03-06-1996
		EP 0554240 A	11-08-1993
		ES 2082862 T	01-04-1996
		JP 5500002 T	14-01-1993
		US 5349127 A	20-09-1994

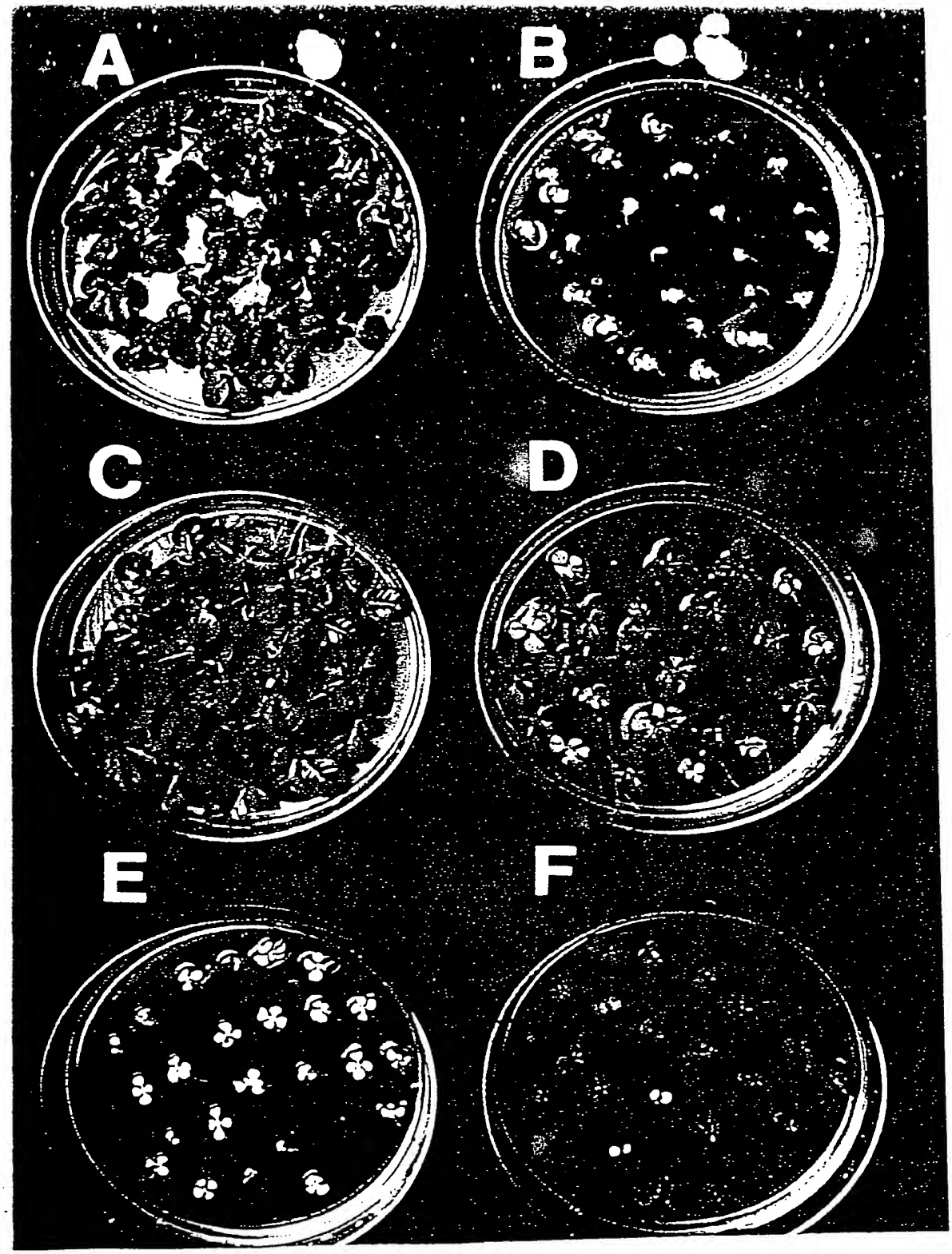

Figure 5

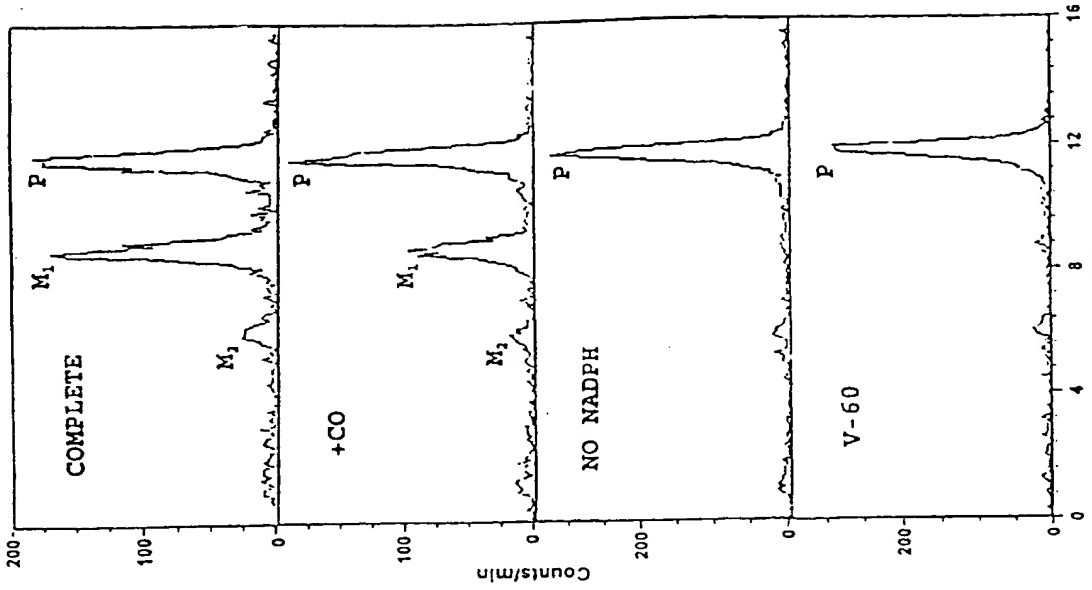

Fig. 4

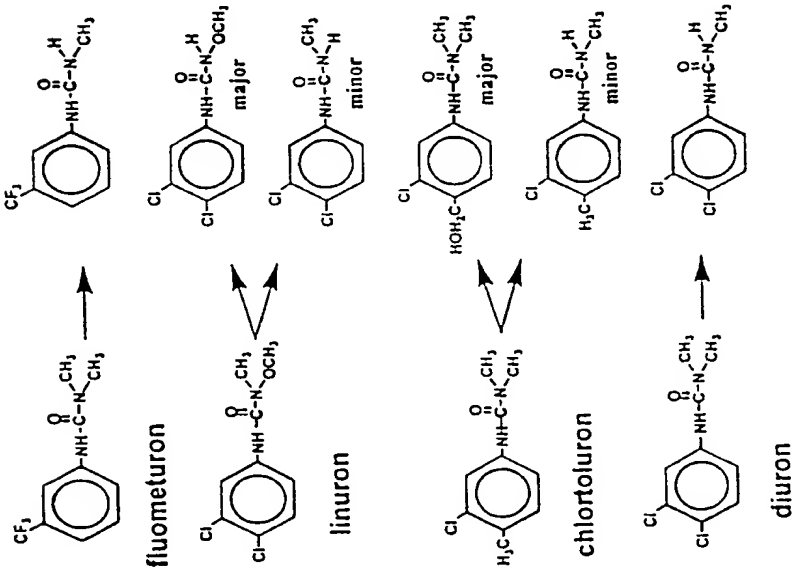

Fig. 5